

INORGANIC CHEMICAL ELEMENTS IN PREPARED MEALS IN SWEDEN

Food collection and Analytical techniques,

Concentration in body compartments,

Metabolic aspects, and

Public health/Clinical significance

MOHAMMED ABDULLA 1986

LUND SWEDEN 1985

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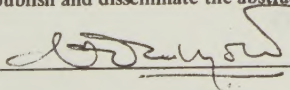
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Abstract The present study deals with the analytical, nutritional and clinical aspects of inorganic chemical elements. For investigating the dietary intake of inorganic chemical elements in small, well-defined groups in the general population, the duplicate portion food sampling technique is a simple, practicable and reliable procedure. The validity of this technique can be verified by repeated studies in the same population as well as by measuring the excretion of the electrolytes, sodium and potassium and nitrogen in 24-hour urine samples. Employing the duplicate portion technique, the concentration of sodium, potassium, calcium, magnesium, iron, zinc, copper and selenium in 882 daily dietary samples from various well-defined population groups in Sweden has been documented. In a limited number of samples, the concentration of chromium, nickel, iodine, lead, cadmium, and mercury has also been measured. The results indicate that the intake of the elements, potassium, magnesium, zinc, copper and selenium in the majority of diets analysed is low when compared with the recommended dietary allowance (RDA) values. Vegetarian diets, however, have higher concentration of these elements than the non-vegetarian diets. The relatively high concentration of raw food materials in the vegetarian diets may be one of the reasons for this difference. On the other hand, it is uncertain whether these elements in the vegetarian diets are biologically available. The concentration of the toxic elements, lead, cadmium and mercury in the diets analysed is far below the permitted levels. Chemical analysis gave lower values than that are obtained by computation using food composition tables. Food tables may not be ideal for the estimation of trace and ultra-trace elements in prepared meals. At present, there are no suitable techniques for the early detection of marginal deficiencies of trace elements. Clinical follow-ups of the pensioners included in the present study for more than 10 years do not reveal any signs or symptoms of a deficiency of any trace elements.			
Key words Duplicate portion food collection technique, Dietary intakes, Sodium, Potassium, Calcium, Magnesium, Iron, Zinc, Copper, Selenium, Iodine, Chromium, Nickel, Lead, Mercury, Cadmium, Public health/Clinical significance			
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Date November 18, 1985

INORGANIC CHEMICAL ELEMENTS IN PREPARED MEALS IN SWEDEN

Food collection and Analytical techniques, Concentration
in body compartments, Metabolic aspects, and Public
health/Clinical significance

AKADEMISK AVHANDLING

av

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B.Sc., Med. Lic. Mö

Offentlig disputation för medicine doktorsgraden
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ERRATTA

- Page 6, line 26, constitutes should read constitute
- Page 11, line 20, anthropometric should read anthropometric
- Page 12, line 4, comprising should read comprised
- Page 21, line 29, are should be read have been
- Page 24, line 18, after similar, add, to
- Page 32, line 17, continuos should read continuous
- Page 32, line 33, 4-5 mols should read 4-5 mmols
- Page 34, line 8, after 20 mmols, delete, <
- Page 39, line 11, diarrhoel should read diarrhoeal
- Page 42, line 25, menopaus should read menopause
- Page 51, line 17, ineficiency should read inefficiency
- Page 57, line 22, selenien should read selenium
- Page 58, line 28, after unabsorbed, add, selenium
- Page 61, line 5, is should be replaced by are
- Page 65, line 19, 40 mg should read 40 μ g
- Page 67, line 13, centration should read concentration
- Page 75, line 21, cobalt-stimulated should read cobalt, stimulated
- Page 87, line 33, after (7-8 μ g), add, /kg body weight
- Page 96, line 9, group should read groups
- Page 97, line 3, protein-calory should read protein-calorie

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IN PREPARED MEALS IN SWEDEN***

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concentration in body compartments, metabolic aspects
and public health/clinical significance

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Lund 1986

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This summary is based on the following publications:

- I. Mohamed Abdulla, Ingrid Andersson, Per Belfrage, Ingrid Dencker, Margaretha Jägerstad, Arne Melander, Åke Nordén, Bengt Scherstén, Thomas Thulin and Björn Åkesson. 1979. Assessment of food consumption: In "Nutrition and Old Age", Scand. J. Gastroent. vol. 14, Suppl. 52 pp. 28 - 41.
- II. Mohamed Abdulla, Margaretha Jägerstad, Thomas Thulin, Sven Svensson and Åke Nordén. 1979. Sodium: idem, pp. 138 - 142.
- III. Mohamed Abdulla, Margaretha Jägerstad, Thomas Thulin, Sven Svensson and Åke Nordén. 1979. Potassium: idem, pp. 143 - 147.
- IV. Margaretha Jägerstad, Mohamed Abdulla, Sven Svensson and Åke Nordén. 1979. Calcium: idem, pp. 148 - 152.
- V. Margaretha Jägerstad, Mohamed Abdulla, Sven Svensson and Åke Nordén. 1979. Magnesium: idem, pp. 153 - 157.
- VI. Mohamed Abdulla, Sven Svensson and Åke Nordén. 1979. Zinc: idem, pp. 168 - 171.
- VII. Mohamed Abdulla and Sven Svensson. 1979. Copper: idem, pp. 172 - 175.
- VIII. Mohamed Abdulla and Sven Svensson. 1979. Chromium and Nickel: idem, pp. 176 - 180.
- IX. Mohamed Abdulla, Kurt Kolar and Sven Svensson. 1979. Selenium: idem, pp. 181 - 184.
- X. Mohamed Abdulla, Margaretha Jägerstad, Arne Melander, Åke Nordén, Sven Svensson and Elisabeth Wåhlin. 1979. Iodine: idem, pp. 185 - 190.

- XI. Mohamed Abdulla, Margaretha Jägerstad, Kurt Kolar, Åke Nordén, Andrejs Schütz and Sven Svensson. 1983. Essential and toxic inorganic elements in prepared meals - 24-hour dietary sampling technique employing duplicate portion technique. In "Trace element analytical chemistry in medicine and biology", vol.2. Editors, P. Brätter and P. Schrammel. Walter de Gruyter & Co. Berlin, New York. pp. 75 - 86.
- XII. Thomas Thulin, Mohamed Abdulla, Ingrid Dencker, Margaretha Jägerstad, Arne Melander, Åke Nordén, Bengt Scherstén and Björn Åkesson. 1980. Comparison of energy and nutrient intakes in women with high and low blood pressure levels: *Acta Med Scand.* 208: 367 - 373.
- XIII. Mohamed Abdulla, Ingrid Andersson, Nils-Georg Asp, Knud Berthelsen, Downen Birkhed, Ingrid Dencker, Claes-Göran Johansson, Margaretha Jägerstad, Kurt Kolar, Baboo Nair, Peter Nilsson-Ehle, Åke Nordén, Solveig Rassner, Björn Åkesson and Per-Arne Öckerman. 1981. Nutrient intake and health status of vegans. Chemical analyses of diets using the duplicate portion sampling technique. *Amer J Clin Nutr* 34:11, 2464 - 2477.
- XIV. Mohamed Abdulla, Karl-Otto Aly, Ingrid Andersson, Nils-Georg Asp, Downen Birkhed, Ingrid Dencker, Claes-Göran Johansson, Margaretha Jägerstad, Kurt Kolar, Baboo Nair, Peter Nilsson-Ehle, Åke Nordén, Solveig Rassner, Sven Svensson, Björn Åkesson and Per-Arne Öckerman. 1984. Nutrient intake and health status of lactovegetarians: Chemical analyses of diets using the duplicate portion sampling technique. *Amer J Clin Nutr.* 40: 325 - 338.
- XV. Mohamed Abdulla. 1983. Public Health/clinical significance of inorganic chemical elements. Nutritional adequacy, nutrient availability and needs. Ed. J. Mauron. Birkhäuser Verlag Basel, Boston, Stuttgart, pp. 338 - 355.

TABLE OF CONTENTS	PAGE
INTRODUCTION	6
AIM OF STUDY	8
STUDY GROUPS	10
METHODOLOGICAL CONSIDERATIONS	14
ANALYTICAL METHODS	22
RESULTS	25
SODIUM	25
POTASSIUM	29
CALCIUM	32
MAGNESIUM	36
IRON	40
ZINC	46
COPPER	51
SELENIUM	56
CHROMIUM	63
NICKEL	65
IODINE	68
MANGANESE	72
ARSENIC, COBALT, MOLYBDENUM	74
SILICON, TIN AND VANADIUM	
LEAD, CADMIUM AND MERCURY	81
PUBLIC HEALTH/CLINICAL	90
SIGNIFICANCE OF INORGANIC	
CHEMICAL ELEMENTS	
CONCLUSIONS	97
ACKNOWLEDGEMENTS	98
REFERENCES	100
PAPERS I - XV	

INTRODUCTION

During the last 20 years, remarkable progress has occurred in many areas of biology and health sciences. Trace element research has definitely shared in this explosion of scientific knowledge. Extensive research conducted in various parts of the world in recent years has clearly demonstrated that many more inorganic chemical elements than previously considered essential play vital roles as structural and functional components of metalloproteins and enzymes in living cells. At the same time, there is growing recognition of the adverse effects of cumulative exposure to heavy metals in small amounts and of "essential" elements in large amounts. Rapid advances in analytical techniques and sophisticated instrumentation introduced during the last few decades have not only helped to recognize a large number of inorganic chemical elements in living systems and their food chain but have also added a new dimension to our understanding of the role of trace elements in health and disease. Recent estimates indicate that most tissues of a living organism contain as many as 40 - 80 inorganic chemical elements (Hamilton, 1979; Abdulla and Dashti 1984b). Although the bulk of living matter consists mainly of the five elements, hydrogen, nitrogen, carbon, oxygen and sulphur, most mammals cannot survive without the so-called "trace-elements" which constitute only less than 0.01 % of the total weight of a living organism.

Hydrogen and oxygen (water) alone constitutes 60 - 65 % of an adult human body. Inorganic chemical elements were earlier classified as "bulk" and "trace" substances of living matter depending upon their concentrations. Between the bulk and trace elements, a third group of elements such as sodium, potassium, calcium, magnesium, chlorine and phosphorous is sometimes termed the macrominerals.

Traditionally, the term "trace elements" is given to a group of inorganic chemical elements such as iron, iodine, fluorine, copper, manganese, zinc, cobalt, chromium, molybdenum, selenium, vanadium and arsenic. The concentrations of these

elements in living tissues and circulating fluids are much lower than that of the macrominerals and as such they could not be quantified accurately until recent years and hence these elements were conveniently termed as trace metals (Underwood, 1977). Today, most elements found in biological materials can be measured accurately and it is being suggested that the inorganic chemical elements should be simply classified as essential or non-essential when they are discussed in relation to health and disease. The number of elements that are found to be of importance in health and disease is likely to be increased in future. Ultimately we may be dealing with all the naturally occurring inorganic chemical elements. At least the trend for such an expansion has been noted during the last few decades.

The nutritional importance of the inorganic elements has grown rapidly during the last century mainly due to better understanding of their biological functions. In deficiency states, most inorganic elements concerned with major metabolic events create health problems. Iron deficiency anemia is a good example illustrating this. In terms of people afflicted, it is second only to protein-calorie malnutrition. Several other pathological conditions in animals, including man, are implied to be associated with deficiencies of essential chemical elements. Although malnutrition and starvation are restricted to certain poverty-stricken areas of the globe, it has become increasingly evident that a marginal deficiency of several essential elements, including zinc, copper and selenium is fairly common even in affluent countries (Mertz, 1970, 1981a, 1981b). Basic biochemical mechanisms by which certain inorganic chemical elements influence metabolism and cause clinical deficiency symptoms in man both primary and secondary, are not yet definitely and completely elucidated. Establishment of the optimum daily requirement and determination of the actual daily intake are important current problems of trace element nutrition. Further, the intake levels that produce either a marginal deficiency or toxicity, especially in vulnerable groups, are still being discussed. In recent years it has been found that the chemical nature of the elements as found in food and the interaction between

each other and with other nutrients play important roles in their absorption and metabolism. Moreover, the importance of supplementation of food stuffs, from a public health point of view, and clinical interest in total parenteral nutrition are current issues of importance. The influence of inorganic chemical elements in the development of malignancy and altered immunocompetence has become another important area of research in recent years.

One of the basic requirements for nutritional research concerned with the inorganic chemical elements is the knowledge of the exposure levels. Barring occupational exposure, the food chain remains the major pathway through which the chemical elements enter the human body. Only limited information is available at present concerning the dietary intakes of the essential and toxic trace elements from prepared meals. Without such information, the public health and clinical significance of trace elements cannot be assessed adequately. The present study is an attempt to tackle this problem.

AIM OF THE PRESENT INVESTIGATION

The present study deals with the actual intake of a number of inorganic chemical elements in the 24-hour diets of various apparently healthy populations in Sweden. The conventional techniques such as diet history and interview studies in combination with food tables employed for the assessment of dietary intakes of micronutrients including inorganic chemical elements are often inadequate. They do not provide information regarding the exact composition of diets served on the plate for individual consumption. The exact data can only be obtained by the direct analysis of the actual diets consumed by individuals. In practical terms, this can only be possible by developing a food collection technique that will provide 24-hour dietary samples which will represent as exactly as possible, the solid foods and fluids normally consumed by healthy individuals. Borgström et al (1970) worked out a technique during the late 60's to assess the dietary intake of fat, especially that of saturated fat and cholesterol in the diet of apparently healthy subjects. The principle of the

technique was to duplicate visually everything that has been consumed by an individual during a 24-hour period. This technique was later standardized and the analysis of proteins and carbohydrates was added in the study of dietary intakes of nutrients. The importance of inorganic chemical elements, especially that of "trace elements" in human nutrition became a very important issue during the early 70's. The information concerning the dietary intake of these elements at that time on the other hand was very scanty. As late as in 1973, Durnin et al emphasized the importance of developing techniques that will provide actual data concerning the intake of nutrients. The present study will attempt to describe the method used in order to estimate the concentration of inorganic chemical elements by direct analysis of 24-hour diets in different population groups in various parts of Sweden. The major part of the present study was carried out at the Unit for Community Care Centre at Dalby, in the southern part of Sweden close to the University town of Lund. The present study will also discuss the adequacy of the intake levels of the inorganic elements analysed in relation to the current recommended dietary allowance (RDA) levels. The study will also describe the significance of a number of minor ("ultra") trace elements that are found in most human diets. The actual concentration of these "ultra" trace elements in the diets studied in the present investigation is, however, not determined due to technical difficulties. Finally, an attempt will be made to correlate the general health status of the populations studied and the intake level of the elements.

STUDY GROUPS

The 24-hour dietary samples were collected from 6 different groups residing in various parts of Sweden.

Group 1. The first group included in the present study concerns 19 apparently healthy individuals (10 men and 9 women) of working age residing in the village of Dalby. They were randomly selected from the telephone directory. The participants who agreed to take part in the study were contacted by a trained dietician who did a survey of the dietary habits of the whole group and explained to the participants the details of the duplicate portion technique. Each subject collected 7 daily dietary samples representing different days of the week. Thus a total number of 133 samples were collected by the first group. Conventional clinical and laboratory tests were done to assess the health status of the participants.

Group 2. The second group constitutes 17 men and 20 women belonging to a group of pensioners born in 1902. During the year 1969, the village of Dalby had a population of 8609 persons and out of this 103 (58 male and 45 female) were 67 years of age. The pensioners were invited to take part in the investigation covering medical, social, odontological and psychological aspects. Because of a number of problems that are described elsewhere (Borgström et al 1979), only 37 subjects participated in the dietary study. As in the case of the first group, the dietician contacted all the participants and explained the purpose of the study and the details of the duplicate portion technique. Once again each participant collected 7 duplicate portions representing different days of the week. In this group 2 subjects (one male and one female) collected only 3 samples each and hence were excluded for calculation of the average intake of specific components. In order to check the health status of the pensioners, clinical and laboratory examinations were performed every two years starting from 1969. The tests are being performed on those who are still alive.

Group 3. The subjects in the third group consisted of 60 women of mean age 52 ± 13.5 years. The subjects were selected from participants in an ophthalmological survey performed in the Dalby population during 1969 - 70 (Thulin et al 1978). From this population of 963 females, 30 with blood pressure above the 95th percentile and a similar age-matched group with blood pressure below the 30th percentile were selected to participate in the study. The main objective of the study was to relate the salt and energy intake to their blood pressure. As in the first two groups, the dietician instructed the participants on the collection procedure and the purpose of the study. The food samples were collected on six days arranged as three groups of two consecutive days during a period of four weeks. During the second day in each of the three periods, 24-hour urine samples were also collected. Urine samples were also collected from the whole group on two other occasions without the simultaneous collection of dietary samples. Apart from the collection of dietary and urine samples, the subjects participating in the study were subjected to anthropometric and blood pressure measurements. A number of biochemical parameters were also included to assess their health status.

Group 4. The fourth group consisted of three healthy men and three healthy women (age 49 - 53 years) who had followed a strict vegan dietary regime for at least three years. The subjects were selected and instructed in how to take part in the study at a health center in the central part of Sweden. The subjects consumed a standardized vegan diet which consisted mainly of fruits, berries, vegetables, roots, nuts and pulses. All animal foods including milk and dairy products were excluded from the diets. Table salt, sugar, pepper, coffee and common tea were also excluded from the menu. Each subject participating in the study was instructed to collect duplicate portions during 4 consecutive days. Thus, a total of 24 daily portions were collected from this group. Apart from the dietary samples, 24-hour urinary specimens were also collected from the whole group corresponding to the days of food collection. At the time of the nutritional investiga-

tion, the participants underwent a medical examination to assess the health status. At the same time blood samples were also collected to determine biochemical parameters.

Group 5. The fifth group comprising 6 middle-aged subjects (3 men and 3 women, 46 - 58 years old) who had followed a strict lacto-vegetarian dietary regime for 29 years (median). They collected 24 dietary samples and a corresponding number of urine samples. The participants were selected from the staff at a lacto-vegetarian health center in the middle of Sweden. Their diets consisted mainly of food items of vegetable origin as well as milk and dairy products. Like the vegans, the lactovegetarians also avoided table salt, sugar, pepper, coffee and common tea in their diets. The subjects in this study also underwent a medical examination at the time of the nutritional investigation to assess the health status. Blood samples were collected at the same time to analyse the biochemical parameters.

Group 6. The final group in the present study consisted of 15 teenagers (7 male and 8 females) residing in the northern part of Sweden. During the week days, the youngsters were living in a hostel mainly due to the great distances to their homes. During the weekends, they normally go home. Once again, the dietician did a dietary survey of the participants' eating habits and gave instruction about the collection of duplicate portions. A total of 75 duplicate portions, 15 urine samples and 15 blood samples were collected from this group. No medical examination was done on this group. None of the participants were suffering from any known diseases.

Most of the subjects who participated in the present investigation were also instructed to make written protocols showing the type and approximate quantities of various food items consumed during the period of food collection. Most groups except the teenagers performed this duty very well. Table I shows a short summary of the various groups included in the present investigation.

Table I. Number, age and sex of the target groups included
in the present investigation

Target Group	Total number		Age	Publication related to this summary
	male	female		
1 - Adults of working age	10	9	25-53	XI, XV
2 - Elderly	17	20	67	I - XI
3 - Females with low and high blood pressure		60	30-80	XII
4 - Vegans	3	3	49-56	XIII
5 - Lactovegetarians	3	3	46-56	XIV
7 - Teenagers	7	8	13	XV

METHODOLOGICAL CONSIDERATIONS

One of the most significant problems in nutrition research on inorganic chemical elements concerns the quantity and availability of these micronutrients in prepared meals. The concentration of these elements in the meals of a defined population can be estimated by direct or indirect methods. Indirect methods which are generally based on statistical data on food production and records of imports and export of food articles give only approximate estimates of macrominerals and often such methods do not provide information concerning the contents of trace elements in food items served on the plate. The methods currently used for the assessment of nutrient composition of diets are listed in table II.

Table II.

Methods used for the estimation of nutrients

1. National food disappearance (Food balance sheets).
2. Household food disappearance.
3. Weighed intake and subsequent chemical analysis from dietary record.
4. 24-hour and 7-day dietary recall.
5. The duplicate portion technique.
6. Calculations based on energy expenditure.

Generally speaking, all the methods mentioned above have limitations. No one method is ideal for application to the problems of trace element nutrition in countries with widely differing resources, life-styles, and diversity of food supply and distribution networks. In a country like Sweden, these problems are, however, limited. Direct methods involving the chemical analysis of the actual food consumed is the only means for the assessment of food components so far not available in standard tables. This is especially true for the trace and ultra-trace metals. The data that is available at present regarding the dietary intake of trace elements is unsatisfactory since most of it is based on conventional techniques, such as diet history and interview studies in

conjunction with the use of standard food tables showing the concentration of various nutrients in (individual) food materials. Usually, such tables are based on the chemical analysis of raw food samples. The nutrient composition of individual food items, especially that of micronutrients such as trace elements may vary in different parts of the world due to seasonal changes and the differences in geographic distribution of the elements in the soil. Further, the concentration of elements such as copper, chromium, manganese and selenium in prepared meals is difficult to compute from the figures in food tables because of lack of such data as well as the low concentration of these elements in various food items. Food tables usually give average values even for macrominerals and they do not take into account the effect of preparing and cooking which alters the composition of raw food materials prior to consumption. Thus, although indispensable for mass production and mass surveillance of food, the data computed from standard food tables may not give a true picture of what the individual is actually eating. Estimates of the requirement of a given food component, presumes a detailed health status analysis of the individual consumer in order to exclude a deficiency state.

The introduction of duplicate portion technique has eliminated a number of problems associated with the computation of data using food tables. The duplicate portion is a copy collected while eating normally to represent the food and fluids consumed during 24 hours by visual judgement. Although slightly inferior to the direct weighing and subsequent chemical analysis method, the duplicate portion is probably the most satisfactory technique by which one can study the actual intake levels of most nutrients. This is specially true for small scale studies aimed at procuring individual consumption data. One of the major criticism directed towards this technique is that people may not maintain their normal eating habits when they are asked to collect a duplicate portion of what they consume during 24 hours. This aspect has been studied in the groups of women, the vegans and the lactovegetarians included in the present study. The input and the excretion in the urine of electrolytes and nitrogen ought to be the same if the subjects are in metabolic balance and if

corrections are made for losses in faeces and sweat (Isaksson, 1980). In the group of 60 women, the urinary volume as well as the electrolytes were measured on three different occasions: once in 1972 and twice in 1975. On the first two occasions, the female subjects were asked to collect 24-hour urine samples only and on the last occasion, they were asked to collect urine samples as well as duplicate portions of their diets. Similarly, the vegans and lactovegetarians also were asked to collect both urine and dietary samples. Tables IIIa and IIIb shows the summary of results.

Table IIIa. Urinary concentration of sodium and potassium in the women of study group III during three different sampling occasions

	year of sample collection		
	1972	1975a	1975b
Number of samples	60	60	60
Urinary volume (ml)	1166	1266	1152
Sodium excretion (mmol/day)	126	136	120
Potassium excretion	63	59	57

Table IIIb. Dietary intake and urinary excretion of sodium and potassium in the subjects participating in the study groups III, IV and V

Element	Group	Intake (mmol/day)	Excretion (mmol/day)
Sodium	III	100	100
	IV	97	87
	V	96	97
Potassium	III	54	57
	IV	103	86
	V	106	87

As can be observed from Table IIa, the urinary excretion of sodium and potassium was virtually unchanged between 1972 and the two sampling occasions in 1975. Similar results were noted by other investigators in Sweden (Karlberg and Tolagen, 1977). By looking at these figures alone, we were satisfied with the fact that the food collection procedure did not

significantly disturb the dietary habits. In Table IIIb, the intake and the excretion of the electrolytes are shown. The input and output of these elements ought to be of the same magnitude when the subjects are in metabolic balance. The intake levels in the subjects of group III are significantly lower than that of the excretion. One reason for this discrepancy was the losses that occurred during the wet-ashing procedure. This was later corrected in the study concerning the dietary intake of trace elements in the food of teenagers. The results in the improvements of the techniques prior to the chemical analysis can also be observed from the figures concerning the intake and excretion of the electrolytes and nitrogen in the subjects in groups IV and V. The validity of the duplicate portion technique was examined in detail in the study concerning the dietary habits of subjects in group V (lactovegetarians). The intake of protein was compared with the urinary excretion of the subjects in this group. Taking into account the average losses that occur through feces, skin and other sources, it was found that the total losses calculated as protein was 52 - 53 g as compared with the intake of 60 g/day. These sort of figures are normally found in subjects living in metabolic wards where optimum facilities are provided for the exact calculation of dietary intakes and excretion of most nutrients. The intake and urinary excretion of electrolytes in the lactovegetarians also did not differ significantly.

In order to check the adequacy of food tables for calculating the concentrations of inorganic chemical elements in food, we analysed 24 hospital diets and the results obtained with regards to calcium and iron were compared with that obtained through a computer using standard food tables. Figures I and II show the correlations. As can be observed, the computed data in general gives higher values than those that are obtained through chemical analyses. The correlations are better when related to macrominerals such as calcium and magnesium than the trace elements. Since the food tables do not have sufficient data for many trace elements, we could not make any comparisons for elements such as zinc, copper, manganese and selenium. Moreover, it is generally accepted at

present that food tables are not suited for the computation of data concerning the dietary intake of most trace elements.

The influence of various procedures employed in the preparation of food samples prior to analysis was also investigated in detail. The representativity and reproducibility of the lyophilisation and extraction procedures were checked by analysing different aliquots of the homogenates and the fat-free powder on different occasions. The correlation between the dry weight of the fat-free and water-free material obtained on two different occasions was excellent (Borgström et al, 1979) The influence of lyophilization and fat extraction on the concentration of the inorganic chemicals was studied by analysing the homogenates directly and comparing the results obtained through the analysis of fat-free lyophilized powder. In most cases, the difference was less than 5 %.

Wet-ashing procedure using concentrated sulphuric, perchloric and nitric acids under 250 °C is the most commonly used technique at present for the determination of most inorganic chemical elements in biological materials. Dry-ashing at temperatures between 450 - 500 °C results in a great loss of several trace elements and as such not recommended at present. If the temperature is kept between 200 - 250 °C during the wet-ashing procedure, the losses of most inorganic elements can be kept to a minimum. We have, however, observed that even when the temperatures are maintained below 250 °C, losses of up to 10 - 15 % were noted in the case of magnesium, sodium and potassium. Further, the wet-ashing procedure is tedious and involves a number of steps before the completion of the digestion. Recently we have tried to minimize the problems involved in the preparation of food samples prior to analysis by hydrolysing the samples in concentrated nitric acid at 70 °C, for five hours (Svensson and Abdulla, 1983). This method was used for the analysis of food samples from teenagers in northern Sweden and a comparison of the results with that obtained through wet-ashing procedure did not differ significantly.

Figure I: Comparison of the concentration of calcium computed using food tables and that obtained by chemical analysis (hospital diets).

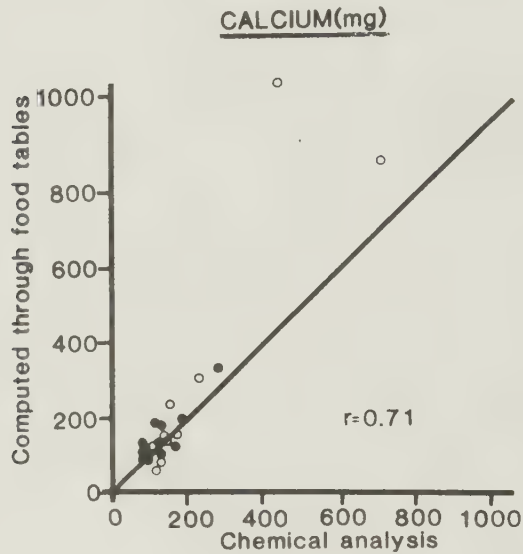
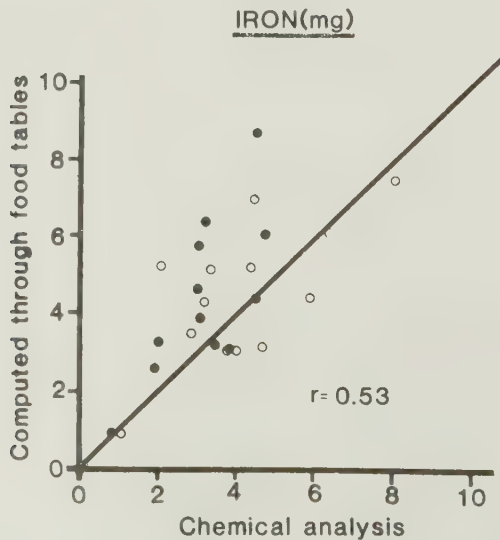


Figure II: Comparison of the concentration of iron computed using food tables and that obtained by chemical analysis (hospital diets).



Only a limited number of studies concerning the inorganic chemical element composition of prepared meals have been done so far in Scandinavian countries. Moreover, such studies have only dealt with a few elements such as iron and calcium. In 1980, Varo et al., compared the mineral composition of Finnish foods obtained through direct analysis and computation using food tables (Varo et al., 1980). They found that the absolute difference between the analytical and computed values was below 10 % for elements with concentration over 5 ppm. The analytical values were lower than the computed figures. The difference became much higher for several trace elements. Persson (1984) investigated the dietary habits of children of different ages in Sweden employing 24-hour recall, 7-day recording of the food intake and dietary history techniques (Persson 1984, Persson and Carlgren, 1985). Iron and calcium were the only inorganic elements included in the study. The iron and calcium intakes were found to be adequate. No iron deficiency anemia was observed in the whole group. Persson and Carlgren (1985) also evaluated various dietary assessment techniques and observed that 24-hour recall and dietary history differed significantly; the dietary history gave much higher estimation of intake levels for many nutrients than the recall method. On the other hand, they found a good agreement between the values obtained through recall and direct analysis of the duplicate portions, especially for the macronutrients (Persson and Carlgren 1985).

The advantages and disadvantages of various methods used for obtaining food consumption data are dealt with by a number of authors in recent years, and it is generally accepted that the duplicate portion technique is probably the most satisfactory technique for studying the dietary habits of small groups in general population (WHO, 1983; Marr, 1971; Bazarre and Myers, 1979; Block, 1982; Burk and Pao, 1976; Young, 1981; van Staveren and Burema, 1985; Callmer et al, 1985).

ANALYTICAL METHODS

A number of methods are currently available for the analysis of inorganic chemical elements in biological materials and food-stuffs. In general, the methods can be classified as follows:

- 1) Calorimetry and spectrophotometry
- 2) Electrochemical methods - a) ion selective electrodes
b) polarography and voltametry
- 3) Atomic emission spectroscopy
- 4) Flame photometry
- 5) Atomic absorption spectroscopy
- 6) Atomic fluorescence spectroscopy
- 7) High temperature atomic emission sources - eg. ICP
- 8) Radioactivation analysis
- 9) X-ray fluorescence spectrometry
- 10) Mass spectrometry

Most of these techniques are normally used for the determination of the mass of an element present in a homogenized sample. For the micro and biochemical distribution of the elements in biological materials, a number of very sophisticated instruments such as the scanning electron microscope and laser probe analysers are currently available.

In the present study, atomic absorption spectrophotometry has mainly been used for the analysis of most inorganic chemical elements. This technique has been widely used for the analysis of trace elements in foods (Wolf, 1983. Ihnat 1981.) For the analysis of sodium and potassium, both flame photometry and atomic absorption spectrophotometry have been used. Analysis of trace elements employing atomic absorption spectrophotometry became very popular during the late 50's and early 60's. The foundation of the technique was laid by Walsh in 1955 (Hamilton, 1979). For the principles of the technique, the reader is directed to a number of standard text books (Hamilton, 1979. Christian and Feldman, 1970).

The sample preparation

After homogenization, fat-extraction and lyophilization, the dry food material was wet-ashed using concentrated sulphuric, nitric and perchloric acids. The wet-ashing was done in 50 ml Kjeldhal flasks. In most cases, 0.5 g of the fat-free lyophilized powder was used for wet-ashing. Initially 0.5 - 1.0 ml of concentrated sulphuric acid and 2 - 5 ml of a concentrated perchloric-nitric acid mixture were added to the food samples and the ashing procedure was continued by adding 1 ml aliquots of the nitric-perchloric acid mixture. The samples were heated after each addition until a slight charring occurred. Normally, 10 additions of the acid mixture was necessary for the completion of the ashing procedure. The temperature during the ashing procedure was kept around 180 - 190 °C. At the end, the excess nitric acid was boiled off. The time required for a complete wet-ashing procedure was approximately 4 - 8 hours. When the procedure was completed, the contents in the kjeldahl flask was diluted to 5 ml with deionized water. For the analysis of calcium and magnesium, the samples were diluted with a solution containing 2 - 3 % lanthanum chloride. For the analysis of cadmium and lead, the digested samples were complexed with dithizone and extracted into a methyl isobutyl ketone phase. The instruments used for the present study were a Varian AA-6 and a Perkin-Elmer 403 atomic absorption spectrophotometers. For the analysis of sodium and potassium, we also used a IL (Instrumental Laboratories) flame photometer. For each element analysed in the present investigation, recovery studies were performed by adding known amounts of the elements in the food samples prior to the wet digestion procedure. Since we did not have a standard reference material for food samples at the time of the present investigation, we had to use our own standards in order to test the validity of the analytical procedure.

The food samples from the teen-agers were wet-ashed by nitric acid alone below a temperature of 100 °C (Svensson and Abdulla, 1983). The hydrolysis was completed within 5 hours in most cases. A comparison of the results obtained using this digestion procedure and the standardized technique used

for the food samples from other age groups did not differ significantly.

The recovery experiments for different elements indicated that the wet-ashing techniques used in the present investigation was very good. In most cases, we obtained a 95 - 100 % recovery of the added elements. In case of sodium and potassium, the losses during the standardized wet-ashing procedure was about 15 %. This was completely eliminated using the mild hydrolysis technique using nitric acid alone (Svensson and Abdulla, 1983). The reproducibility of the analytical method using atomic absorption spectrophotometer was very good and the variation coefficient in most cases was less than 5 %.

A number of food samples were also analysed by neutron activation technique and the results obtained were compared with that obtained by atomic absorption spectrophotometer. The results were in close agreement. We also sent a few samples to other laboratories in Europe for trace elements analysis and the results obtained were similar that found in our laboratory (Abdulla et al, 1985d). On the whole, the sample preparation technique and the instrumental methods used in the present investigation were the same as those currently in use by a number of laboratories throughout the world.

RESULTS

A. Sodium

An average adult human body contains about 4 mols of sodium which is equivalent to around 230 g of common salt. A little more than half of this is in the extracellular fluid and most of the remaining part is deposited in the bone as inorganic sodium salts. Only a small amount of the total body sodium is found in the intracellular space. During changes in the sodium balance, plasma concentrations are kept relatively constant through regulation of the exchangeable sodium in the skeleton. Total exchangeable sodium in a healthy adult has been found to be 40 mmol/kg using the Na^{24} isotope dilution technique (Davidson et al., 1979).

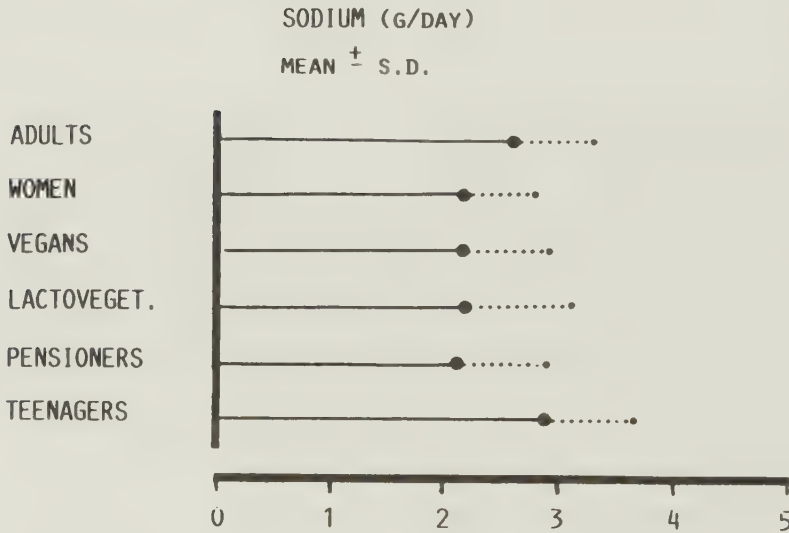
Normal daily diets in the industrialized countries contain 5 - 18 g of salt equivalent to 80 - 300 mmols of sodium. Prepared meals served for consumption seldom contain more than 10 - 12 g of salt. The major source of sodium in common meals is the table salt used in cooking and seasoning. Industrial processing of individual food items and nutritional additives may also contribute to the total contents of salt in the average daily diets. In addition to the salted foods, many of the common food items such as bread, cheese, milk, eggs etc contain considerable amounts of sodium. In most affluent countries, snack foods also contribute significant amounts of sodium as common salt to the daily intake.

When in metabolic balance, the input and output of sodium are equal. The input is from the food and extra table salt added to the meals only while the output is mainly in the urine. Small amounts are excreted in the feces and occasionally, significant amounts of sodium are lost in sweat. The intake of sodium is generally calculated from the urinary excretion levels.

Figure III shows the intake of sodium in the groups included in the present investigation. The average intake was around 2 - 2.5 g sodium/day which is equivalent to 5 - 6 g common

salt. According to the latest recommendations by the National Academy of Science, USA, an intake of 6 - 9 g sodium as common salt is considered to be adequate (RDA, 1980). The sodium content in the diets of the vegans and lactovegetarians was almost the same as that found in the nonvegetarian diets although the first named groups usually avoid the use of table salt.

Figure III: Concentration of sodium in the diets of various population groups included in the present investigation.



Although there exists considerable controversy with respect to the role of sodium in the etiology and prevention of cardiovascular diseases, a number of epidemiological, experimental and clinical studies have shown that the degree of excess salt intake is closely related to the development of hypertension (Guang Sheng and Chai, 1982; Pilkänen, 1982). In this context, two distinct questions may be posed. Does a high salt intake cause hypertension and conversely, can salt reduction be recommended as a means of therapy in cardiovascular diseases. With respect to the first question, a number

of studies have been done in primitive populations with low salt intake. In most of such studies, low systolic and diastolic blood pressures have been observed throughout the average life span of the groups investigated (Williams 1980). In countries such as Japan and Finland where the salt consumption is high, the mortality from cardiovascular diseases is also high (Puska et al, 1982; Sasaki, 1982; To Chikubo et al, 1982). Recent studies (Marx, 1981) have added further evidence for the relation between excess salt in the diet and hypertension. According to these new results, excess salt in the diet may indirectly produce hypertension by causing the release of a hormone, called natriuretic hormone, which is found in kidney and other tissues of the body (Marx, 1981). This hormone acts on the sodium transport in such a way that if susceptible individuals eat more salt than their kidney can handle, their blood pressure is increased, mainly through the contraction of smooth muscles in the arterioles (Marx, 1981). This theory explains, to some extent, the genetic basis of hypertension related to salt intake in susceptible individuals.

In our study of the Dalby women (III), we have tried to study the dietary habits of women with high and low blood pressure, especially with regards to the intake of salt. Table IV shows the blood pressures and the sodium intake of the two groups of women with high and low blood pressures.

As can be observed from the table, the mean values for the intake of sodium did not differ significantly between the two groups despite the large difference in blood pressure. This finding does not justify restriction of salt intake alone as a means for decreasing blood pressure in the population.

The intake of sodium in the vegans and vegetarians is almost the same as that of the other groups investigated although salt is not used in the preparation of vegan and vegetarian foods. The blood pressure levels were normal or slightly low for their ages when compared with that of the nonvegetarians. Once again, a clear relation between the salt intake and blood pressure cannot be drawn from the results of this study.

Urinary excretion of sodium and potassium has been widely used for the calculation of the intake levels whenever direct chemical analysis is not possible. These values can also be used for the assessment of the validity of the duplicate portion technique. As explained earlier, we have investigated this matter in detail and we do not find that the subjects included in the present study deliberately deviated from their normal dietary habits when asked to collect the duplicate portions.

Table IV The intake of sodium and blood pressure in two groups of women (study group III).

Study groups	Blood pressure mm Hg	Sodium intake mmol/day
Group I. Women with high blood pressure	$179 \pm 32^*/97 \pm 13^{**}$ $p < 0.01$	105 ± 31 (ca 6 g salt)
Group II. Women with low blood pressure	$123 \pm 19^*/73 \pm 7^{**}$ *systolic **diastolic	NS 94 ± 25

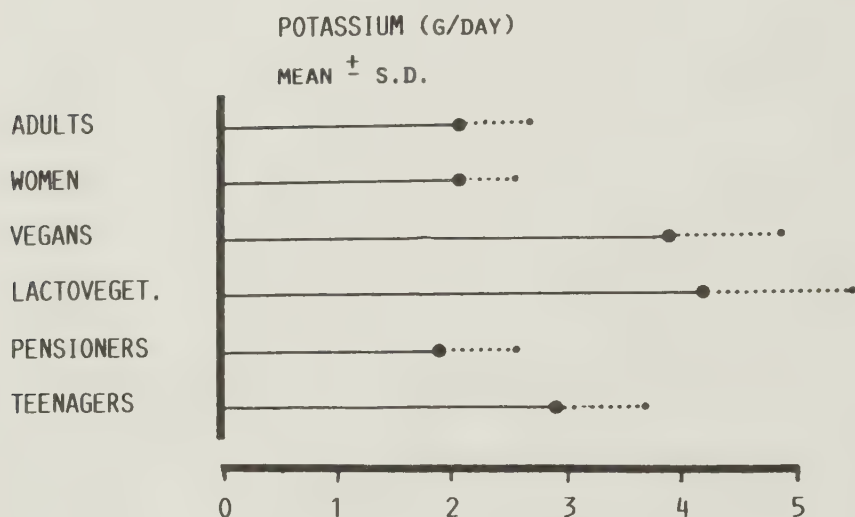
B. Potassium

An average human adult body contains 3.5 mmol (140 g) of potassium. It is the principle cation of intracellular fluid and nearly 98 % of the total potassium in the body is located in the intracellular space. The rest is found in the inter cellular space and plasma. Bone contains only a negligible portion of the total potassium. The total body potassium is somewhat low in females and declines with increasing age. The total potassium in the body can be measured through whole body counting using ^{40}K isotope. It can also be measured as exchangeable potassium by the isotope dilution technique using the isotope ^{42}K (Laurell et al 1980). It is generally assumed that an average Western diet contain about 70 - 100 mmol of potassium daily. An average intake of 100 mmol (4 g) potassium/day is more than adequate to meet the daily requirement. The absorption of potassium from the daily diet is almost 100 % and during a steady state, 90 % of the absorbed potassium is excreted via the kidneys. The rest of it is lost in feces and desquamated epithelium. Increased amounts of potassium are lost in urine, whenever there is tissue damage. Breakdown of cellular proteins, starvation and trauma are some of the common causes of potassium depletion mediated through an increased excretion in urine. In most healthy tissues, 1 g nitrogen is associated with approximately 2.7 mmol of potassium and the potassium/nitrogen ratio in the tissue is often a very useful index indicating the loss or gain of cellular mass.

Figure IV shows the intake of potassium in the various groups included in the present study. The average content in non-vegetarian diets is around 50 mmol/day. The vegetarian diets on the other hand had a relatively higher amount of potassium than the common diets. The diets of the Vegans were particularly high in potassium. The potassium contents in fresh green vegetables are known to be higher than that found in processed foods (Abdulla et al, 1980b; Underwood, 1977). The diets of the Vegans and Lactovegetarians included in the present study did have a high percentage of green vegetables and fruits. Fish, meat and milk products are also good sour-

ces of potassium. The consumption of potatoes and milk - two staple food items in Sweden - in most of the groups included in the present study was normal except in the group of Vegans.

Figure IV: Concentration of potassium in the diets of various population groups included in the present study.



Potassium deficiency induced mainly through an increased consumption of highly refined food articles with low potassium concentrations has been reported in parts of the populations of most industrialized countries during the last two decades (Judge 1972, 1974; Abdulla et al 1975). An average Western diet is estimated to contain 50 - 100 mmol of potassium. In Japan, the daily dietary intake has been found to be 50 mmols (Pitkänen 1982) which is almost the same as that found in the diets of non-vegetarians included in the present study. When the dietary intake is reduced, potassium deficiency develops slowly (Judge 1972). It takes a few weeks before the kidney can adjust to increases or decreases in the potassium load. In contrast to the capacity of the kidneys to

produce saltfree urine during situations that precipitates a pure sodium deficiency, the conservation capacity of the kidneys for potassium is rather poor (Judge 1972, Davidson 1979). Although a pure potassium deficiency is rare, it has been observed that certain vulnerable groups such as the elderly and alcoholics are more at risk than the population in general (Abdulla et al 1977, Abdulla and Dashti 1984b). Studies conducted by Judge (1972) indicate that potassium deficiency is fairly common in parts of the populations of affluent countries. Balance studies conducted in human subjects indicate a safe intake of 65 mmol/day (Judge 1972). If one accepts this figure which is also in accordance with US recommendations (RDA 1980), the intake of potassium from the diet of non-vegetarians, especially that of pensioners included in the present study is low. Traditionally, potassium status in the body is judged by the plasma or serum levels. Looking at the plasma levels of the pensioners potassium intake appears to be normal. Unfortunately, plasma level of potassium and other intracellular chemical elements is a very poor indicator of potassium status (Abdulla 1983b, Editorial BMJ, 1967). The most reliable indicator of potassium status is whole body counting as mentioned earlier. This technique, however, is unavailable in most clinics. Due to a lack of characteristic symptoms and diagnostic techniques, marginal deficiency of potassium can seldom be diagnosed at an early stage. Long-term clinical follow-up of vulnerable groups in the general population is probably the ideal way to detect the deficiencies of inorganic chemical elements including potassium. This procedure, however, is expensive and time-consuming. In developing countries, the highest priority is given to the prevention of malnutrition concerned with macronutrients such as proteins. In the present study, we are following up the health status of the pensioners every two years until their death. So far, we have followed the surviving pensioners for 12 - 14 years and judging from the general health and levels of potassium in the plasma, there are no signs of potassium deficiency in them. The plasma levels of potassium in the other groups investigated in the present study are also within the reference range. It appears that the present recommendations are higher than the minimal

amount required for maintaining a balance. It is also possible that the human body can adapt to a lower intake level than recommended for maintaining this balance. Our present data, on the whole, regarding the dietary intake of potassium together with the clinical parameters showing normal functions of most of the subjects investigated indicate that the amount measured in the diets are adequate to meet the daily requirements.

C. Calcium

Depending on height, an average male human adult body contains 25 - 30 mols (1 000 - 1 200 g) of calcium. Females have somewhat lower amounts than males (20 - 24 mols; 770 - 920 g) Cohn et al, 1976; American Academy of Pediatrics, 1976). In general, calcium constitutes 1.3 - 1.8 % of the total adult body weight. Most of the calcium in the body (almost 99 %) is localised in the skeleton and as such the biggest depot for calcium. There is a continuous exchange of calcium between the skeleton and the rest of the body. According to Whedon (1964), 18 mmol (700 mg) of calcium enter and leave the bones daily. About 25 mmol (1 g) of the total body calcium is in the cells and 50 mmol (2 g) in the extracellular space. Serum calcium constitutes approximately 15 % of the total calcium found in the intravascular space (7.5 mmol; 300 mg) (Laurell et al 1980). Smaller amounts of calcium (1.2 mmol/l) in the body fluids is in ionized form and this is essential for blood coagulation, neuromuscular activity, enzymatic and regulatory processes, cell permeability and synthesis of new bone. The serum calcium levels are under homeostatic control and are regulated by the parathyroid hormone, calcitonin, metabolites of vitamin D and the dietary calcium.

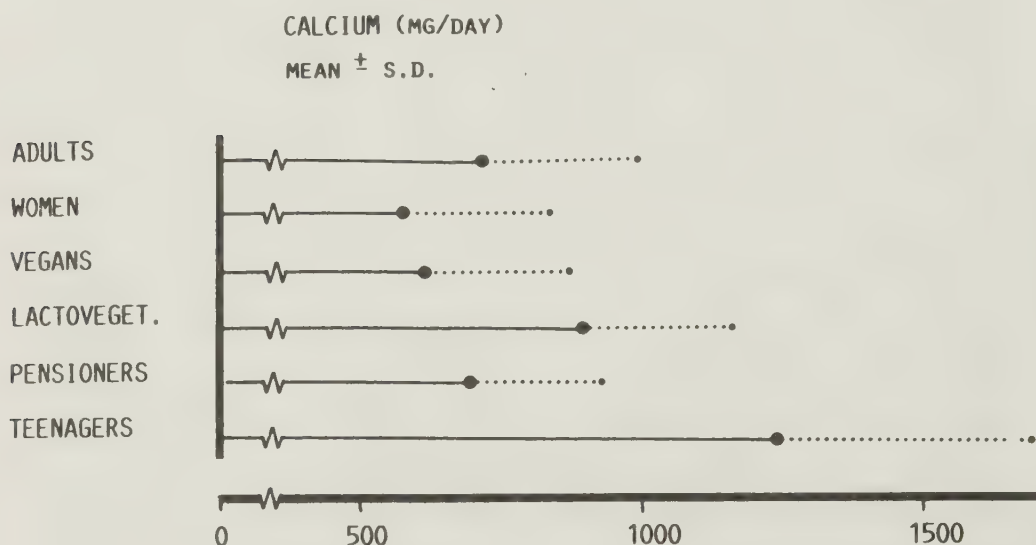
The dietary intake of calcium varies greatly in different parts of the world. Populations in many developing countries thriving on 4 - 5 mols (150 - 200 mg) of calcium per day seem to have no problem with their bone or teeth. In affluent countries, the calcium intake is high mainly due to the consumption of milk and dairy products which are rich in calcium. In general, the intake levels exceed 500 mg per day.

Only small amounts of the ingested calcium is absorbed and the major part of the dietary calcium is excreted in the feces. The dermal losses are estimated to be 15 - 20 mg/day. Calcium absorption depends on a number of factors including proteins, lactose, phosphate, vitamin D, phytate, fiber, fat and oxalic acid. The absorption takes place mainly in the duodenum and upper part of jejunum through passive diffusion and active transport through the mucosa. In recent years, a calcium-binding protein has been isolated from the jejunal mucosa of several animal species and man (Morrisey et al, 1975). The synthesis of this protein is partially dependent on vitamin D. During active growth, the synthesis of this protein is greatly increased. The decrease in calcium absorption observed in the elderly has been thought to be due to a decrease in the synthesis of this protein as well as an impaired synthesis of $1,25(\text{OH})_2\text{D}_3$ by the kidney (Jägerstad 1982). Under normal circumstances, 15 - 40 % of the dietary calcium is absorbed. Most adults can adapt to a low intake by an increased absorption (Nicolysen 1943). When the demand is high during conditions such as pregnancy and lactation, the absorbed calcium is utilised effectively and the breakdown of the skeleton decreases. Parathyroid hormone, calcitonin and the capacity of the kidney to transform vitamin D to the active metabolite play important roles in this adaptation process (Ribivich and de Luca 1978). Studies by Hegsted et al (1952) showed that calcium balance could be maintained with a daily intake as low as 3 - 5 mols (100 - 150 mg) of calcium.

Figure V shows the intake of calcium in the diets of the population groups included in the present study. The average intake in most of the diets analysed is around 20 mmol (800 mg). In Sweden, milk and other dairy products provide 75 % of the dietary calcium except in the diet of the vegans. Almost half of the calcium intake originates from milk alone. The relatively high amounts of calcium in the vegan diets are probably due to the high consumption of nuts and other food items of vegetable origin which are exceptionally rich in calcium. The lactovegetarian diets on the other hand were very rich in dairy products and the calcium levels were higher than most of the other diets analysed (XIV). The

highest amount of calcium was found in the diet of teen-agers (1 200 mg/day).

Figure V: Concentration of calcium in the diets of various population groups participated in the present study.



The FAO/WHO Expert Group (1972) recommends an intake of 10 - 12.5 mmols (400 - 500 mg) of calcium per day for adults. The Food and Nutrition Board in USA (1974) on the other hand suggested an intake of 20 mmols < (800 mg) for adults. The Swedish recommendation is at present > 15 mmols per 1 000 kcal which is rather high (Kost och Motion 1975). During pregnancy and lactation, most countries recommend a daily intake of 25 - 30 mmols (1 000 - 1 200 mg) (Zollner et al 1977). In the study group investigated in the present study, pregnant and lactating women are not included. On the whole, our results showing the intake levels and clinical picture including the biochemical data suggest that the dietary intake found is adequate to maintain the balance.

It is wellknown that there is a relationship between the protein intake and calcium metabolism. The relation between calcium and protein seems to be important with regards to calcium excretion in urine. The urinary excretion of calcium

is directly related to the protein intake (Johnson et al 1970, Walker et al 1972, McCance and Widdowson 1942, Margan et al 1974, Chu et al 1975). This increase is thought to be mediated through an increased glomerular filtration followed by an inhibition of tubular reabsorption. Normally, 100 - 350 mg calcium is excreted in the urine (Davis et al 1970). There is, however, a great individual variation. Women after the menopause have an increased excretion of calcium in the urine (Nordin 1971). Menopausal osteoporosis can be partially explained on the basis of this increased excretion. Urinary excretion of calcium is decreased when the protein intake is low. On a moderately high protein intake, the calcium losses exceed the quantity absorbed. This phenomenon is important in developing countries where both protein and calcium intakes are much lower than in affluent countries. The ratio between the dietary protein and calcium usually provides a good index with regards to the bioavailability of calcium. In the study of the pensioners, we found a good correlation between intakes of calcium and protein (IV). A high calcium diet on the other hand may interfere with the absorption of other divalent cations such as zinc, magnesium, iron and copper (Underwood 1977). Prolonged consumption of diets rich in calcium may induce a secondary deficiency of the above metals if sufficient amounts of such metals are not present in the diets to counteract the adverse effects of calcium. (Abdulla et al, 1977; Anonymous 1960). In laboratory animals, administration of diets rich in calcium has been shown to have a protective effect on the toxicity of heavy metals such as lead and cadmium (Morrison et al 1977, Quarterman and Morrison 1975). During the last two decades, a number of epidemiological and clinical studies have shown correlations between calcium contents in the drinking water and mortality from cardiovascular disease. Most of the results, however, had been conflicting and inconclusive. The protective effects of calcium against cardiovascular diseases have been indirectly correlated to the interaction of calcium and toxic metals such as lead and cadmium (Revis et al 1981, Crawford et al 1968). Most of the epidemiological data had been derived from studies concerned with mortality rates in "soft" and "hard" water areas. From the limited groups in-

cluded in the present report, it is difficult to draw any conclusions concerning the protective effect of calcium as mentioned above.

D. Magnesium

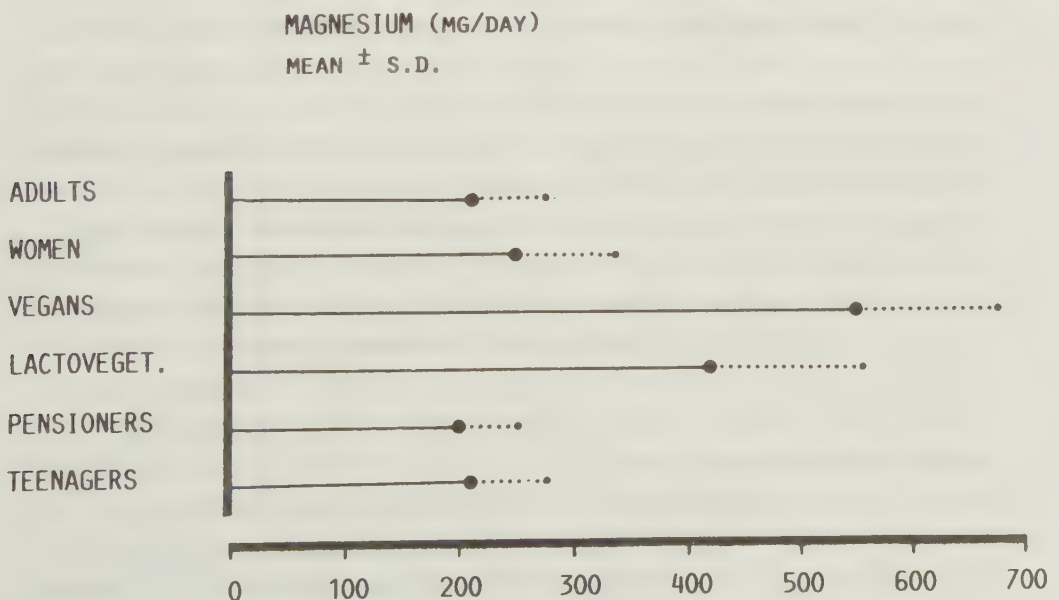
The limited data available from analysis of human carcasses indicate that an average adult contains around 1 000 mmol (25 g) of magnesium. This amount is equally distributed between the skeleton and intracellular space. The average contents in the bone is estimated to be 45 mmol/kg wet weight (Aikawa 1981). Only a negligible part of the total magnesium is found in the extracellular space (about 10 mmol). Among the other body tissues, the muscles have the highest magnesium concentration (6 - 11 mmol/kg) (Aikawa, 1981; Szelényi 1973). Next to potassium, magnesium is the prominent cation in the cells. The contents of magnesium in the plasma is around 0.9 mmol/l of which 65 % is in the ionized form and the rest is protein-bound. Magnesium is a co-factor to a number of important enzymes and it is necessary for protein synthesis. It is also involved in carbohydrate metabolism. Oxidative-phosphorylation requires the presence of magnesium. Similar to calcium, magnesium has an important role in neuromuscular contraction.

The ingested magnesium is absorbed both from jejunum and ileum. Approximately 25 - 35 % of the dietary intake is absorbed. Although an active process, the intestinal absorption of magnesium does not require a specific factor as in the case of calcium. The absorption on the other hand is inversely correlated to the intake similar to calcium. The excretion of magnesium is mainly via the kidney through glomerular filtration followed by partial tubular reabsorption. During deficiency states, the urinary excretion of magnesium can be as low as 0.5 mmol/day. The tubular reabsorption under such conditions can be as high as 99 per cent. Most of the unabsorbed magnesium is excreted in the feces. Considerable amount of magnesium can also be excreted in sweat. Acclimation does not occur, as in the case for sodium and potassium (Aikawa 1981). There are a number of

similarities in the metabolism of potassium and magnesium. Changes in the metabolic state of the body and the pH can alter the concentration of magnesium in the intra and extra-cellular spaces, as is observed in the case of potassium during similar circumstances. The homeostasis of magnesium is influenced by parathyroid hormone, aldosterone and thyroxin (Laurell et al 1980; Aikawa, 1976, 1981).

Figure VI shows the concentration of magnesium found in the diets of various groups included in the present study. The mean concentration of magnesium in non-vegetarian diets (common diets) is around 8.5 mmol (200 mg)/day. The vegetarian diets on the other hand contained twice as much as in the common diets. This is probably due to the high percentage of green vegetables and cereals in the vegetarian diets which are rich in magnesium. On the other hand, refined food items which constitute a considerable proportion of the common diets, are poor in magnesium. This factor alone may explain the low magnesium contents observed in the common diets.

Figure VI: Concentration of magnesium in the diets of various population groups participated in the present investigation.



As in the case of other inorganic chemical elements, there exists considerable controversy regarding the minimum and adequate requirements (MDA and RDA) of magnesium in man. Recommendations vary with an intake between 6 - 14 mmol (140 - 340 mg)/day (Jones et al 1967, RDA 1980, FAO/WHO 1973, Nicolysen 1969, Aikawa 1981). A number of dietary components are known to influence the absorption of magnesium. High intakes of protein and calcium may decrease the absorption of magnesium (Bunce et al 1963, Colby and Frye 1951, Varo 1974, Flink 1976, Aikawa 1981).

The results of the present investigation indicate that the magnesium contents in the common diets consumed by the majority of people in Sweden is low when compared with the recommended dietary allowance (RDA 1980) levels. When expressed as mmol/1000 kCal, it is about 10 % lower than that recommended by FAO/WHO (1973). The high contents of refined food articles in the diet as well as the cooking procedures may contribute to the reduction of magnesium, as explained earlier, but some losses (5 - 10 %) have also occurred in the wet-ashing procedure of the dietary samples prior to analysis.

Until very recently, it has been thought that a pure magnesium deficiency hardly ever occurs in man. Recent studies, however, indicate that there is such a condition (Flink 1956, Wacker et al 1962, Aikawa 1981). Pure magnesium deficiency is associated with a number of clinical features including muscular tremor, vertigo, ataxia, tetany and convulsions (Hanna et al 1960). A number of clinical conditions are known to be associated with depletion of magnesium in human adults. Cardiovascular diseases, diabetes, diseases of the gastrointestinal tract, liver cirrhosis, chronic alcoholism and treatment with diuretics for prolonged periods are some of the conditions which may precipitate a secondary magnesium deficiency (Iseri et al 1975, Aikawa 1981). The clinical signs of magnesium deficiency, especially that of marginal deficiency may be difficult to diagnose at an early stage (Abdulla 1980a). Levels of magnesium in plasma, similar to that of other intracellular cations may be a poor indicator of magnesium status. A normal serum/plasma magnesium level does not

exclude magnesium deficiency (Abdulla 1980a, Aikawa 1981). Since the magnesium in the plasma is largely regulated by the kidneys, urinary output after a magnesium loading test may provide useful information concerning the magnesium status (Thorén 1963). If tissue reserves are adequate, more than 80 % of an i.v. administered magnesium dose is excreted in the urine (Thorén 1971). Measurements of magnesium in muscle and lymphocytes may in some cases add information concerning the body status of the element.

A few vulnerable groups in the general population including children with prolonged diarrhoeal disease, the elderly with continuous diuretic treatment, pregnant women and alcoholics are more likely to be affected by marginal deficiency of magnesium than others. Alcoholism has increased considerably in the West during the last few decades and alcoholic liver cirrhosis is one of the leading cause of death in affluent societies today (Sherlock 1984, Dashti et al 1985). In a preliminary study of 100 chronic alcoholics admitted to the psychiatric hospital in Lund, we found low magnesium levels in the plasma of 25 % of them (unpublished). Magnesium substitution is not normally practiced in Swedish hospitals. A fall in serum/plasma magnesium is associated with a transient decrease in the concentration of potassium and coincides with the onset of neuromuscular hyperexcitability that characterizes the withdrawal state (Mendelson et al 1959, Wolfe and Victor 1969, Aikawa 1981). Pregnant women who consume even moderate amounts of alcohol are more at risk than non-alcoholics. Even normal pregnancy is associated with a subclinical deficiency of magnesium (Aikawa 1981). Longterm follow-up and responses to therapeutic supplementation with magnesium of the vulnerable groups mentioned above are ways of detecting covert magnesium deficiency.

In the present study, we have clinically followed the pensioners for a period of 12 years and we have not detected any overt signs of magnesium deficiency, although their intake levels are below the recommendations. As long as there are uncertainties regarding the minimum dietary allowance (MDA) of magnesium, it is, indeed, difficult to draw any

conclusions from the present study. It appears that most of the individuals included in the present investigation have intake levels necessary to meet the requirements. In future, it is important, however, to be on the alert for detecting subclinical deficiencies in risk groups.

E. Iron

Traditionally, iron is one of the classical elements that belong to the group of the so-called "trace elements". This element has been known to mankind for centuries. The ancients believed that iron was of heavenly origin. The Greeks and Romans considered iron as a gift from Mars (Schapira 1964). From the time of Sydenham and Willis in the 19th century to the 20th century, the importance of iron was closely linked with the treatment of chlorosis (Schapira 1964, Underwood 1977). Frodisch in 1922 demonstrated that iron concentration in the blood of chlorotics was lower than that of healthy controls (Underwood 1977). The presence of iron in tissues was first demonstrated in 1713 by Lenney and Geoffy (Schapira 1964). In 1886, Zinoffsky estimated the iron content of horse hemoglobin (Underwood 1977). Following this important discovery, attention was focused for many years on the role of iron in the formation of hemoglobin. During the late 19th and early 20th centuries, a number of studies were carried out to establish the importance of iron in human nutrition.

Among the micronutrients, iron is probably the most important and abundant element. It is involved in a wide range of biochemical reactions in the living cell. When coupled with porphyrin and inserted into an appropriate protein, iron not only bind oxygen reversibly but also participates in a number of vital oxydation-reduction reactions. Myoglobin, peroxidase and catalase consist of distinctive proteins bound with the same porphyrin as is found in the haem of haemoglobin. Cytochrome and cytochrome-oxidase are other iron-porphyrin-protein complexes. Myoglobin serves as a small tissue oxygen reserve for the needs of very vigorous muscular activity. Peroxidase and catalase are involved in the scavenging of oxidative substances such as hydrogen peroxide. The cyto-

chrome system is concerned with the oxidation processes and electron transfer.

The total iron content of the human body varies with age, sex, nutrition and state of health. A normal healthy adult weighing 70 kg contains 70 - 90 mmol (4 - 5 g) of iron (Underwood 1977). Haemoglobin accounts for 2/3 of the total body iron. Myoglobin and enzymes containing haem or non-haem constitute 5 - 6 % of the total iron. About 18 mmol (1 g) of iron is stored as ferritin and hemosiderin mainly in the liver.

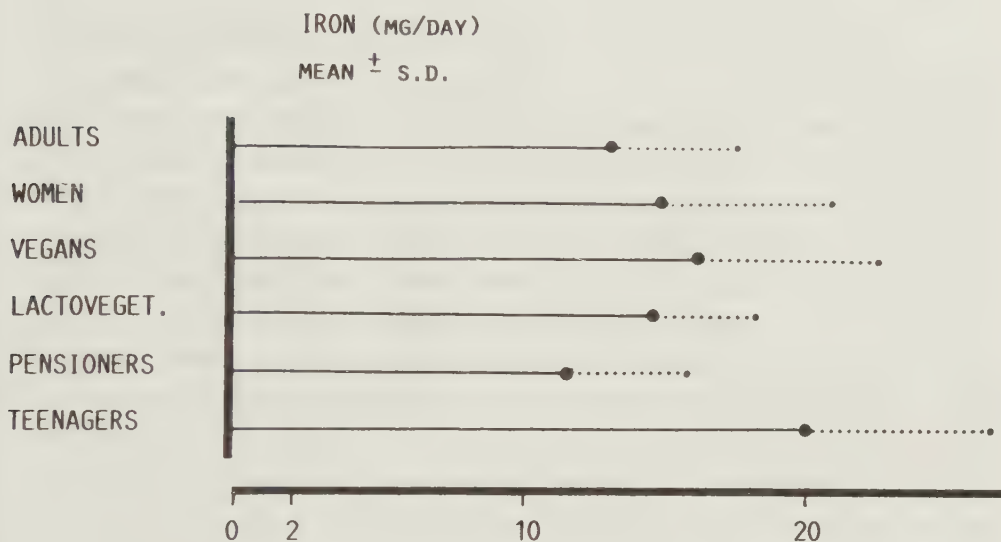
Absorption of dietary iron takes place mainly in the duodenum. A number of factors are known to affect the absorption of iron. This include sex, age and iron status of the individual, chemical form of the ingested iron, and other nutritional components, especially the divalent cations. The mechanism of iron absorption has been intensively investigated since the findings by McCance and Widdowson (1937) that the absorption is quantitatively regulated by body needs. Under normal circumstances, only 5 - 15 % of the iron present in the diet is absorbed. Most of the unabsorbed iron is excreted in the feces. Only very little of the absorbed iron is excreted in the urine. Balance studies using radio isotopes indicate that the true excretory iron is 0.2 - 0.5 mg/day (Dubach et al 1955). This iron is mainly derived from desquamated cells and from the bile. Biliary excretion of iron is estimated to be around 1 mg/day and this originates mainly from the haemoglobin breakdown. Most of this iron is reabsorbed. The mean urinary excretion of iron by healthy adults is 0.2 - 0.5 mg/day (Hawkins 1964). Apart from the excretion in feces and urine, iron is also lost through sweat, nail and hair cuttings. The average loss of iron through sweat in healthy subjects under normal conditions has been estimated to be 0.5 mg/day (Mitchell and Edman 1962). Thus, the total quantity of absorbed iron lost in feces, urine and sweat amounts to 0.6 - 1.0 mg/day in an average healthy individual. Females in the fertile age lose 0.5 - 0.8 mg iron/day (calculated on the basis of a 28 day cycle) as a consequence of menstrual cycle in addition to the normal losses.

Figure VII shows the intake of iron in the diets analysed in the present study. The mean intake in all the groups investigated was above 216 μmol (12 mg)/day. The vegetarian diets had an average concentration of 270 μmol (16 mg)/day. A number of female subjects participated in the study had iron intake below the recommended level of 180 μmol (10 mg)/day. None of them, however, manifested any signs of anemia. The haem iron contents of the diets consumed by the pensioners were 8.5 μmol (0.5 mg)/day. This constituted only 4 - 5 % of the mean intake of total iron. The haem iron concentration in the diet is mainly dependent upon the proportion of individual food items rich in it such as blood sausages, liver etc. The proportion of such components in the menu of most subjects in the present study was low. As can be expected, the vegetarian diets have almost no haem iron.

A number of recent studies have suggested that dietary iron is absorbed from two pools: haem iron and non-haem iron (Hallberg and Björn-Rasmussen 1972). Studies by Björn-Rasmussen et al (1974) have shown that an average adult absorbs 18.1 μmol (1 mg) iron from the diet. The estimated daily loss is very close to the amount absorbed. The absorption increases when the diet contains high amounts of haem iron (Food and Nutrition Research Report 1974).

The recommended daily intake of iron for adult men and for women after menopause is 180 μmol (10 mg), (RDA 1980). The corresponding figure for women in fertile age is 320 μmol (18 mg)/day. Although the diets analysed in the present study in most cases provide adequate amounts of iron, it is, however, difficult to say anything about the bioavailability. The amount of iron that is available for absorption depends primarily on the amount released by the digestive process. Less than half of the total iron in the diet is released by peptic digestion in the stomach and most of this is in the form of ionizable inorganic iron, except the small amounts released from meat as haem complexes (Jacobs 1973).

Figure VII: Concentration of iron found in the diets of various population groups included in the present investigation.



From a nutritional point of view, iron deficiency is one of the most important public health problems. In numbers of people afflicted, it is next to protein-calorie malnutrition. There have been several proposals for and investigations of iron fortification programmes, particularly in developing countries, where the prevalence of iron deficiency anemia is high. Strategies to fortify or enrich food consumed by susceptible populations on the other hand have met considerable opposition from physicians who consider that the risk from iron overload outweighs the benefits of supplementation. A review some years ago concerning food fortification with iron by Finch and Monsen (1972) showed that an increase in the dietary load of iron from 300 to 400 μmol (16.5 to 25 mg) did not result in an increase of the absorbed fraction. The efficacy of iron absorption is also facilitated by the presence of proteins and amino acids which either stimulate acid secretion or by forming amino acid chelates of iron (Martinez-Torres and Layrisse 1973, Elwin 1974, Jacobs 1973). Fortification of flour with iron may not be beneficial. In Sweden, 43 % of the food iron in 1973, however, originated

from fortification (Westin 1975). Recently fortification of milk and common salt with iron had considerable success against iron deficiency anemia in Mexico and India (Rivera et al 1981, 1982; Narasimha Rao 1984, 1985).

Iron status is usually judged by measuring transferrin saturation, concentration of serum/plasma iron, red cell protoporphyrin and serum/plasma ferritin. Deviations of these parameters from the normal values, however, do not always indicate a change in iron status (Cook et al 1976). When all the above parameters are abnormal, iron metabolism is always deranged. Cook et al (1976) investigated the iron status of a proportion of a large population by measuring three of the above (transferrin saturation, red-cell protoporphyrin and serum ferritin) parameters and found that when all the three were abnormal, 63 % of the subjects had anemia. Finch emphasized the importance of using parameters that reflect iron status objectively in population surveys (Cook et al 1972). Serum ferritin is one such parameter. Values less than 10 ng/l in most cases indicate very low body stores of iron (Laurell et al 1980, Qvist et al 1980). In the study of the pensioners included in the present report, none had values below 10 ng/l in their plasma (Qvist et al 1980).

Iron deficiency anemia is very often defined according to the decrease in hemoglobin levels. After iron supplementation in vulnerable groups, hemoglobin levels are usually followed. Hemoglobin levels, on the other hand, may not always explain symptoms because of the adaptation of oxygen transportation and release mediated through 2,3-diphosphoglycerate. A number of studies concerned with iron supplementation in vulnerable groups in the general population such as pregnant women and blood donors do not provide basis for iron therapy (Elwood et al; 1967, Lieden 1974, Qvist et al 1983, 1985).

Summing up the experience from the present study regarding iron intake and the clinical follow-up of the pensioners, there are no indications for suspecting a nutritional iron deficiency in the populations investigated. Whenever iron deficiency is suspected and before starting a supplementation

programme, it is important to look for any pathological conditions other than a pure nutritional deficiency. Increasing the food iron intake by supplementation programmes may in some cases have the unfortunate effect that the diagnosis of a serious pathological bleeding condition is delayed. Strategies of fortification and supplementation in vulnerable groups in the developed as well as developing countries must be based on accurate information concerning the adequacy of different diets and bioavailability with respect to iron. In affluent countries, the trend at present is to consume diets providing low energy with a conservatism in the choice of meals and food components. This practice may create problems in the future (Hallberg 1983). Results of studies in India and Mexico mentioned earlier may throw some light on the beneficial effect of iron supplementation in developing countries.

F. Zinc.

Zinc is probably one of the most important and interesting inorganic elements related to health sciences. Just over a hundred years ago, the biological significance of the element was shown by Raulin (1869). In 1905, Mendel and Bradley showed that zinc is a constituent of the respiratory pigment of the snail. A few years later, the work of Birckner indicated a functional role of zinc in higher animals (Birckner 1919). Somner and Lipman in 1926 observed that zinc was essential for plant life. During the early 1930's, the element was also shown to be essential for growth and wellbeing of rats (Todd et al 1934). An animal disease related to zinc deficiency was described by Tucker and Salmon in 1955. Later, O'Dell et al (1958) showed that zinc is essential for the growth of chicken. The essential role of zinc in human health was described during the early 1960's by Prasad et al (1961), Pories and Strain (1966) and others (Halstead et al 1972, Sandstead et al 1967, Ronaghy et al 1974).

The total body content of zinc in an adult human subject is 30 - 38 mmol (2 - 2.5 g) which is half the body content of iron and much more than the corresponding figures for any other trace element (Widdowson et al 1951, Underwood 1977). The highest concentration of zinc in the body is found in the choroid of the eye and the prostate. Human seminal fluid has 100 times the concentration of plasma/serum. Relatively high concentrations are present in the skin. In animals covered with hair or fur, a considerable amount is found in these tissues. In man, nearly 1/5 of the total body zinc is estimated to be present in the skin (Pories et al 1966). Most of the body zinc is found in the bone at a concentration of 3 $\mu\text{mol/g}$ (200 $\mu\text{g/g}$) compared with an average of 0.5 $\mu\text{mol/g}$ (30 $\mu\text{g/g}$) in fat-free tissues of the rest of the body.

In blood, zinc is present in the erythrocytes, leukocytes and platelets. The concentration of the metal in erythrocytes is estimated to be 0.2 - 0.25 $\mu\text{mol/ml}$ (10 - 15 μg) (Prasad et al 1965). Nearly 80 % of the total blood zinc is confined to the erythrocytes. The leukocytes contain 3 % of the total

zinc in human blood. The concentration of the element in platelets is estimated to be 10 μmol (70 $\mu\text{g/l}$) of whole blood. Human blood plasma/serum contains 12 - 17 μmol (800 - 1 100 $\mu\text{g/l}$) (Davidson et al 1979, Prasad et al 1978, Abdulla et al 1973b, 1978, Abdulla and Svensson 1979a, Qvist et al 1983). Almost all of the zinc in plasma is either loosely or tightly bound to one of several proteins. The principal zinc-binding protein is an α_2 macroglobulin. Nearly 40 % of the total plasma zinc is bound to this protein which is not exchangeable (Parisi and Vallee 1970, Laurell et al 1980). Smaller portions of zinc in the plasma may also bind to proteins such as transferrin (Prasad and Oberleas 1970). Almost 60 % of the total zinc in the plasma is loosely bound to albumin and as such functions as the main transport protein. Less than 10 % of the plasma zinc is bound to amino acids such as histidine and cysteine (Prasad and Oberleas 1970, Giroux and Henkin, 1972).

As mentioned earlier, zinc is crucial to growth, development and normal functions of all living systems. Keilin and Mann (1939, 1940) first demonstrated that zinc is an integral part of the enzyme carbonic anhydrase of the red blood cells. Since then, the metal has been found to be an essential component of many enzymes in the liver, pancreas, kidney and many other organs of man and animals. The importance of this element may be surmised from the fact that both DNA and RNA polymerases are zinc metalloenzymes. During the last few decades, over 90 enzymes dependent on zinc for their activity have been found in various tissues of different animal species (Prasad 1976, 1978; Abdulla and Haeger-Aronsen 1971; Abdulla 1980d). Zinc enzymes participate in a wide variety of metabolic processes including carbohydrate, lipid, protein and nucleic acid synthesis or degradation (Prasad 1982).

Experimental zinc deficiency in animals results in a number of abnormalities, including congenital malformation, growth retardation, abnormal bone metabolism, atrophy of the testes and deformative changes in the skin (Hurley 1985, Apgar 1972, Mathur et al 1978). Zinc-deficiency hypogonadism, associated with dwarfism, hepatosplenomegaly and geophagia in man is a

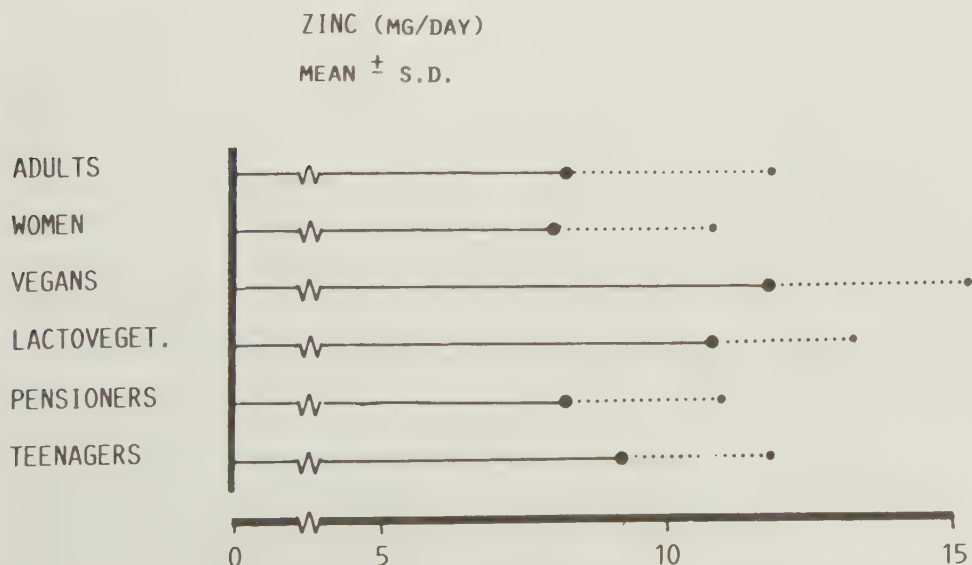
well-established syndrome today. In patients with leg ulcers, impaired healing is related to zinc deficiency (Hallböök and Lanner 1972, Haeger et al 1972). Zinc also plays a key role in the treatment of acrodermatitis enteropathica (Moynahan 1974, Michaelsson 1974). Our own recent findings indicate that zinc may have some protective effect against the action of heavy metals (Abdulla 1980d; 1982c; Abdulla and Haeger-Aronsen 1971, 1974a; Abdulla and Svensson 1979c, Abdulla et al 1979f) Haeger-Aronsen et al 1976, Webb 1972). In vitamin A metabolism, zinc may have a possible role (Smith et al 1973, Abdulla 1974b). The metal also has been found to have a role in experimental and human cancer (Mathur et al 1978, 1979; Abdulla et al 1979b, 1980c, 1982d, 1984a). It has also become evident that zinc is needed for cell-mediated immunity (Prasad 1982, Good et al 1982, Chandra et al 1985, Alexander et al 1982). Zinc may also be involved in the pathophysiology of liver disease (Dashti et al 1985, Abdulla et al 1985a, Sherlock 1984).

Like many other "trace elements" such as iron and copper, only a small percentage of the dietary zinc is absorbed. The absorption takes place mainly in the duodenum and it is affected by a number of factors. Age, size of the individual, the level of dietary zinc, the nutritional status, the presence in the diet of antagonizing substances such as calcium, phosphate, fiber, phytate and chelating agents are the major factors affecting zinc absorption. Among the antagonizing substances, the major inhibitor of zinc absorption is phytate, which is present in many cereals and vegetables (O'Dell and Savage 1960, O'Dell 1969, Reinhold et al 1973, Abdulla and Nordén 1973c). Approximately 20 - 30 % of the ingested dietary zinc is absorbed depending upon its bio-availability (Prasad 1982). Most of the unabsorbed zinc leaves the body through feces. The fecal zinc also includes small amounts (1.5 - 3 μmol , 1 - 2 mg/day) of endogenous zinc secreted via pancreas (Prasad 1982). The urinary excretion of zinc in healthy adults is very low when compared with the intake levels. The normal urinary excretion is 1.5 - 14 μmol (100 - 900 $\mu\text{g/day}$) (Roman 1969), but rises markedly in nephrosis, diabetes, porphyria and alcoholism (Fairhall and Hoyt

1929, Pidduck et al 1970, Peters 1960, Halstead and Smith 1970, Weismann et al 1976). Loss of zinc via sweat is normally low, but may be considerable under hot climatic conditions. Under normal conditions, approximately 5 - 7 μmol (0.5 mg) zinc is lost via sweat. An individual secreting 5 l of sweat/day on the other hand may lose as much as 50 - 70 μmol (5 mg) zinc (Underwood, 1977).

Figure VIII shows the concentration of zinc found in the diets included in the present study for analysis. The average intake in the common diets is around 120 μmol (8 mg)/day. The contents in the vegetarian diets are slightly higher than that of the other diets. This is probably due to the higher proportion of unrefined food items in the vegetarian diets than in the common diets. Modern methods for food processing are accompanied by a reduction of trace elements compared with the natural material. Whereas raw wheat contains 450 μmol (30 mg)/kg wheat flour contains only 150 μmol (10 mg)/kg of zinc. The menu of most of the subjects included in the present study did not show the presence of components rich in zinc such as oysters and other sea delicacies.

Figure VIII: Concentration of zinc in the diets analysed in the present investigation.



Zinc in the normally consumed food of an adult in affluent countries has been estimated to be about 150 - 230 μmol (10 - 15 mg)/day. According to older studies, a daily intake of 153 μmol (10 mg) was recommended, whereas recent studies have shown a somewhat higher requirement of 230 - 300 μmol (10 - 15 mg/day (Mertz 1974). Until recently, only little information was available on the zinc contents in the prepared meals of human subjects. In 1973, the FAO/WHO (1973) expert committee provided data concerning provisional requirements of dietary zinc intake for the first time. This was followed by recommendations from the US National Academy of Sciences (1974). According to their latest recommendations, the requirements are 3 - 5 mg for infants up to the age of 1, 10 mg for children under 10 years of age, 15 mg for adolescents and adults and 20 - 25 mg for women during pregnancy and lactation. Recent studies using isotope techniques have indicated a metabolic requirement of 4 - 6 mg/day (Smith et al 1983, Lowry et al 1981). If these values are considered accurate, then the dietary zinc would have to be 10 - 15 mg considering a 30 - 40 % absorption. Many dietary surveys during the last few years have shown a main daily intake of less than 15 mg without any overt signs of zinc deficiency (Sandström et al 1980, Sandstead et al 1967, Schroeder et al 1967). Most of the recommended figures have, however, not considered the important question of bioavailability.

Several studies in industrialized countries have indicated that pregnant women are not able to receive the recommended levels of zinc by eating the common diets. A majority of the pregnant and lactating women in affluent countries are only receiving 50 % of the recommended levels (Hunt et al 1979, Moser et al 1982). Low zinc intake together with low serum zinc levels in fertile women is known to be associated with complications during pregnancy and at partus (Jameson 1976, Hambidge et al 1972, Cavdar et al 1980). There exists considerable controversy concerning the zinc status and pregnancy at present. In a recent study of pregnant women with low plasma zinc values as reported by Jameson (1976), we did not observe any complications during pregnancy or at partus (Qvist et al 1985). When pregnancy is associated with di-

seases such as diabetes mellitus, the risk for complication is high (Jameson 1983, Abdulla et al 1983c). It appears that detailed studies of a large number of female subjects in developing and developed countries are necessary for elucidating the effect of zinc on pregnancy in humans.

At present, there is no single laboratory parameter that indicate the body stores of zinc. Because of the simplicity and low cost, plasma or serum zinc levels are traditionally used for the assessment of zinc status. A wide range of factors are known to influence plasma/serum zinc and as such may not be a good indicator of zinc status (Abdulla 1983b).

The clinical follow-up of the pensioners included in the present study have shown that the subjects are in good health in spite of the low zinc intake. Plasma levels are within the normal range. With advancing age, there is a reduction in energy metabolism and a deterioration in a number of physiological functions. Digestive inefficiency may occur in the elderly. Many of them are very often under treatment with drugs and as such the requirement of zinc may be higher than the healthy ones. Summing up from the experience of our study of the pensioners in Dalby, the intake levels of 120 μmol (8 mg) of zinc/day appears to be adequate. Marginal deficiencies which is hard to detect may, however, exist in vulnerable groups such as the elderly in affluent countries.

Copper.

The presence of copper in biological materials was observed during the early nineteenth century. The element was considered of no importance to biological systems until 1926 when Mc Hargue suggested its values in the diet of rats (Underwood 1977). Two years later, Hart et al (1928), provided the first evidence that copper plays a key physiological role in vertebrates. Hart and his colleagues demonstrated that copper in addition to iron was necessary for blood formation in rats. In 1934, Cohen and Elvehjem showed that copper is essential for the elaboration of heme A, a component of cytochrome oxidase. During the early thirties, it was

observed in a number of countries that cattle grazing in pastures poor in copper developed anemia. In other species, osteoporosis or ataxia may develop due to copper deficiency (Davidson et al 1979). Copper was later on found to play a key role in the formation of aortic elastin in animals (Underwood 1977).

Mann and Keilin isolated the first cuproprotein from bovine erythrocytes in 1938. Since then, a number of copperprotein compounds were isolated from living tissues of various animal species. These compounds were subsequently shown to be uniquely important in catalyzing the reduction of molecular oxygen to water.

The average adult body contains 1.3 - 1.9 mmol (80 - 120 mg) of copper (Underwood 1977, Cartwright and Wintrobe 1964a, 1964b). In comparison with the amounts of iron and zinc, the copper content in the human body is about 20 - 50 times lower. About one third of the total body copper is found in the liver and brain. In the newborn, hepatic copper is several times higher than in adults (Williams 1982). Redistribution of this high hepatic copper takes place during the first four years of life (Bloomer and Lee 1978). Exceptionally high concentrations of copper occur in the pigmented part of the eye (Underwood 1977). Skeletal muscle due to its high mass contains about one third of the total body copper (Mason 1979).

Copper in blood occurs partly in erythrocytes and partly in plasma. In erythrocytes, the metal is bound to erythrocuprein (Super oxide dismutase, SOD) at a concentration of 54 nmol (3.4 µg/mg) of the protein. Nearly 60 % of the red cell copper is bound to this protein. This protein found in the erythrocytes and other cells eliminates the superoxide (O_2^-) radical. The rest of the erythrocyte copper is loosely bound to nonspecific proteins (Underwood 1977). In the plasma, nearly 90 - 95 % of the copper is bound to ceruloplasmin and this fraction is very stable. The rest of it is loosely bound to albumin. The equilibrium of copper in the intra and extra-cellular fluids is brought out by the albumin-bound copper.

Ceruloplasmin with a molecular weight of 130 000 daltons has a very intense blue colour and each molecule contains six copper atoms (Laurell et al 1980). About 150 mg ceruloplasmin (10 μmol copper) is synthesized in the liver/day. Plasma copper levels are slightly higher in females than in males. Normal values are around 16 μmol (1 mg)/l which is almost the same as that of iron and zinc.

As mentioned earlier, copper is a constituent of many vital enzymes, including cytochrome oxidase, dopamine beta hydroxylase, urate oxidase, tyrosinase, lysyl oxidase and superoxide dismutase (O'Dell 1976, Holwerda et al 1976). In a number of animal species, copper is found to be essential to such diverse activities as heme synthesis, connective tissue metabolism, bone development and nerve function. Copper deficiency in experimental animals results in anemia, abnormalities of hair and skin pigmentation, loss of skeletal and vascular integrity and an impaired immune response (Czarnecki and Kritchewsky 1980). Wilson's disease and Menke's kinky hair syndrome are two wellknown hereditary disorders related to disturbed copper metabolism. In Indian childhood cirrhosis, excessive dietary copper has been postulated to have an etiological role (Abdulla 1979e). Increased copper accumulation is also found in primary biliary cirrhosis and prolonged extrahepatic biliary tract obstruction.

The ingested copper is mainly absorbed from the duodenum in man. Various estimates indicate that about 30 % of the dietary copper is absorbed (Underwood 1977, Williams 1982). The absorption of copper may proceed through an active transport mechanism coupled with amino acid transport or through a carrier-mediated mechanism (Kirchgessner and Grassman 1970, Crampton et al 1965). Metallothionein is thought to be involved in the absorption of copper (Evans et al 1970). The absorption of copper is antagonized by a number of dietary components including ascorbic acid, divalent cations such as zinc and cadmium and phytate (Hunt et al 1970, Van Campen and Gross 1968, Underwood 1977). The major part of the unabsorbed copper appears in the feces. The normal excretory pathway is via the bile. Only small amounts

are lost in the urine, skin and hair. The urinary excretion is estimated to be between 0.16 - 0.96 μmol (0.01 - 0.06 mg)/day (Cartwright and Wintrobe 1964a).

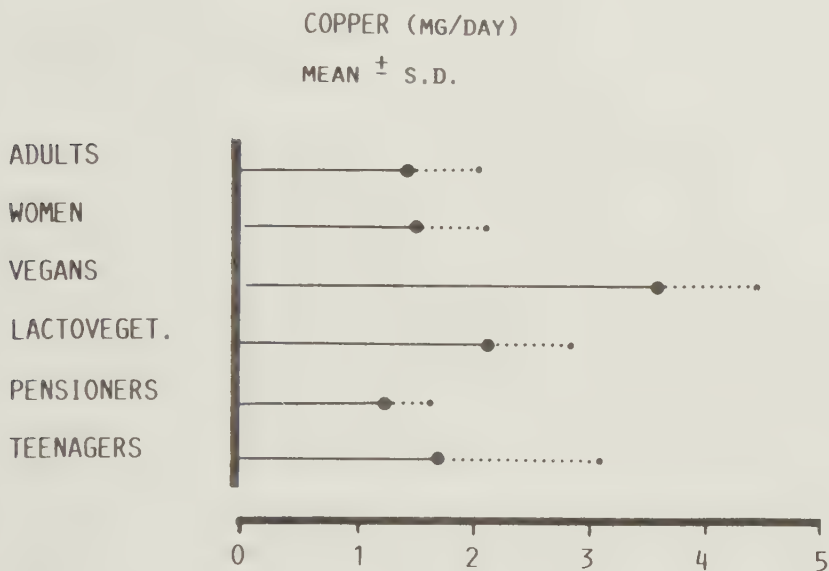
Figure IX shows the concentration of copper in the diets of the various groups included in the present report. The mean concentration of copper in the diets consumed by the non-vegetarians (common diets) was 18 - 27 μmol (1 - 1.5 mg)/day. The vegetarian diets on the other hand contained 3 - 4 times the levels of the common diets. The high content of natural food materials (unrefined) in the vegetarian diets is probably the main reason for this difference. Data available from USA and other industrialized countries show intake levels in the common diets very close to our values (Klevay 1984). A recent report indicate that nearly 80 % of the diets consumed by average healthy adults in most affluent countries contain copper levels less than 24 μmol (1.5 mg)/day (Klevay 1984).

Copper requirements have been measured in human subjects employing balance techniques in metabolic wards. Cartwright summarized several balance studies and concluded that the average requirement is close to 32 μmol (2 mg) (Cartwright 1950). A study by Klevay et al (1980) showed values very similar to that of Cartwright. These estimates are at present considered as reliable and as such represent the minimum daily allowance (MDA) levels. Based on the above figures, the National Academy of Sciences in USA (Food and Nutrition Board) and the WHO recommended that the dietary intake of copper should exceed 32 μmol (2 mg)/day (Williams 1982).

As can be observed from the results of our work as well as from others (Klevay et al 1979), the intake levels from the prepared meals are low when compared with the recommendations, except in the case of vegetarian diets. In India, vegetarian diets based on rice provided an average intake of 70 μmol (4.5 mg)/day (De 1949). These figures are very close to the concentrations found in the diets of the vegetarians included in the present study. In a recent paper, Williams commented on the copper balance as follows: "Thus, it appears that although diets in most settings provide adequate amounts

of copper for copper balance, there may be insufficient copper provided in special diets or in unusual dietary situations, regardless of the country of origin" (Williams 1982).

Figure IX: Concentration of copper in the diets analysed in the present study



A pure nutritional deficiency of copper is rare in human adults. Cordano et al (1964) described cases of anemia in malnourished children who had low copper and ceruloplasmin levels in plasma. These children developed neutropenia as well as anemia which did not respond to iron therapy alone. On the other hand anemia was corrected when the children were treated with copper supplementation. Recently Mason (1979) reported cases of clinical hypochromic anemia and neutropenia in humans associated with copper deficiency. Copper deficiency may also occur in patients receiving prolonged parenteral nutrition (Danford 1984). In such patients, anemia, leukopenia and neutropenia are observed which are reversed by oral copper therapy. Long-term treatment with zinc in humans may induce secondary copper deficiency (Prasad et al 1978, Abdulla 1979d, Abdulla and Svensson 1979a, 1982e; Hoogenraad et al 1979). In Menke's disease, intestinal copper absorption

is impaired and there is a reduction in hepatic copper (Danks et al 1972, Horn and Jensen 1980). Infants with this disorder usually respond to intravenous copper administration with regards to plasma copper and ceruloplasmin levels. Children afflicted with this disease usually die before they are three years old, but copper therapy may prolong their lifespan.

In the present study, we have not observed any signs of copper deficiency in the individuals participating. Although the common diets consumed by the majority of subjects contained copper levels below the recommended figures (32 - 78 μmol , 2 - 5 mg/day) none of them were clinically in negative copper balance. Plasma copper levels were within the normal range in all subjects. The least amount of dietary copper/day that is necessary to maintain the balance is around 20 μmol (1.3 mg) (Klevay et al 1980). Most of the diets analysed in the present series had copper levels above this critical value. The present recommendation of 32 - 78 μmol (2 - 5 mg) may be a little too high. It may, however, be necessary in the future to look for marginal deficiencies of copper in vulnerable groups due to changes in the dietary habits. Metal-ion interactions in the gut is another important aspect to be considered when deficiencies and toxicities of elements such as iron, zinc and copper are discussed. The relatively high intake of calcium, for example, may aggravate the low copper intake by inhibiting the absorption.

Selenium

Selenium was discovered by the Swedish scientist Berzelius in 1817. This element was initially considered only on the basis of its toxicity. Already in 1842, Japha described the toxicity of selenium in animals (Underwood 1977). Almost a century later, two diseases in the livestock caused by selenium toxicity were identified (Rosenfeld and Beath 1964, Underwood 1977). About three decades ago, Schwarz and Folz (1957) could show that liver necrosis in vitamin E deficient rats responded positively to treatment with traces of selenium in the diet. After this important discovery, considerable attention was focused upon the physiological role of

selenium. During the late 1950's, it was also observed that a number of diseases in animals related to vitamin E deficiency, such as white muscle disease, hepatosis, alopecia and infertility responded to vitamin E-selenium therapy. Towards the latter part of 1960's, it was equivocally established that selenium is an essential micronutrient in animals (McCoy and Weswig 1969, Thopson and Scott 1970). In the meantime, studies were in progress with regards to whether selenium was essential in man. McKeehan et al (1976) could show that the growth of human fibroblasts in culture was influenced by selenium. Almost at the same time, Awasti et al (1975) purified glutathione peroxidase from human erythrocytes and found that 3.5 gram atoms of selenium was present per mol of the protein. In 1979, Van Rij et al reported positive effect of selenium supplementation in a patient on long-term parenteral nutrition with muscular discomfort. Finally, the importance of selenium in human nutrition was shown in the studies relating the effect of selenium supplementation in children with cardiomyopathy (Keshan disease) in Peoples' Republic of China (Chen et al 1980).

There is no data at present regarding the exact concentration of selenium in the human body. Tissue concentration of selenium is usually around 0.1 mg/kg (Heydorn 1979). Total body concentration in a healthy human adult is estimated to be 60 - 90 μmol (5 - 7 mg). The kidney cortex contains the highest concentration of selenium followed by the liver and thyroid tissues (Burk 1976). In one study concerning the total contents of selenium in the body, the lowest concentration was found in the fat tissue (Underwood 1977). Selenium in blood is distributed partly in erythrocytes and partly in plasma. Oh et al in 1974 showed that 75 % of the ovine red cell selenium is in the enzyme glutathione peroxidase. Plasma selenium is bound mostly to nonspecific alpha and beta globulins although some of it is also bound to glutathione peroxidase (Dickson and Thomlinson 1967, Noguchi et al 1973). Analysis of blood selenium in 253 Canadians showed 2.3 μmol (182 μg)/l in whole blood, 3 μmol (236 μg)/l in cells and 1.8 μmol (144 μg)/l in plasma respectively (Dickson and Thomlinson 1967). Allaway et al (1968) found mean values of 2.6 μmol

(206 μg)/l selenium in whole blood of subjects living in seleniferous areas. In 1972, Kasperek et al found a mean value of 1.2 μmol (98 μg)/l selenium in the serum of 184 subjects. In general, the concentration of selenium in whole blood varies greatly throughout the world, from below 0.25 μmol (20 μg)/l in China where Keshan disease is prevalent to over 3.8 μmol (300 μg)/l in subjects living in parts of Venezuela where the soil is rich in selenium. In New Zealand, Finland and Sweden, where the soil is poor in selenium, the blood levels in general population are around 0.6 - 1.2 μmol (50 - 100 μg)/l.

The dietary selenium is rapidly and efficiently absorbed from the gastrointestinal tract depending upon the amount and chemical form of the element (Brown et al 1972). Studies using radioisotopes indicate that the duodenum is the main site of absorption in animals. Studies conducted in New Zealand indicate that while selenium in the form of selenomethionine is absorbed almost completely, only 60 % is absorbed when the ingested selenium is in the form of selenite. Recently Christensen et al (1983) showed that selenium in poultry meat is better absorbed than selenite. The absorbed selenium is converted usually to selenide and incorporated in proteins as selenocysteine and selenomethionine. Selenide also can be converted to dimethylselenide which has a garlic-like smell. At high dietary intakes of selenium, the dimethylselenide formed can be lost through exhalation and the garlic-like smell can easily be detected. The absorbed selenium is excreted mainly via the urine as trimethylselenium. The unabsorbed is excreted in the feces. Smaller amounts are also lost in sweat (Schroeder et al 1970, Burk 1976).

As mentioned earlier, there are clear indications at present that selenium is an essential nutrient for humans. The only known function of selenium is in the enzyme glutathione peroxidase which acts as an anti-oxidant and thus protects the biomembranes. A number of animal studies have shown that selenium interacts with vitamin E (Jenkins and Hidiroglu 1972). Several diseases in animals including liver necrosis, striking pallor, and the degeneration of the skeletal muscle,

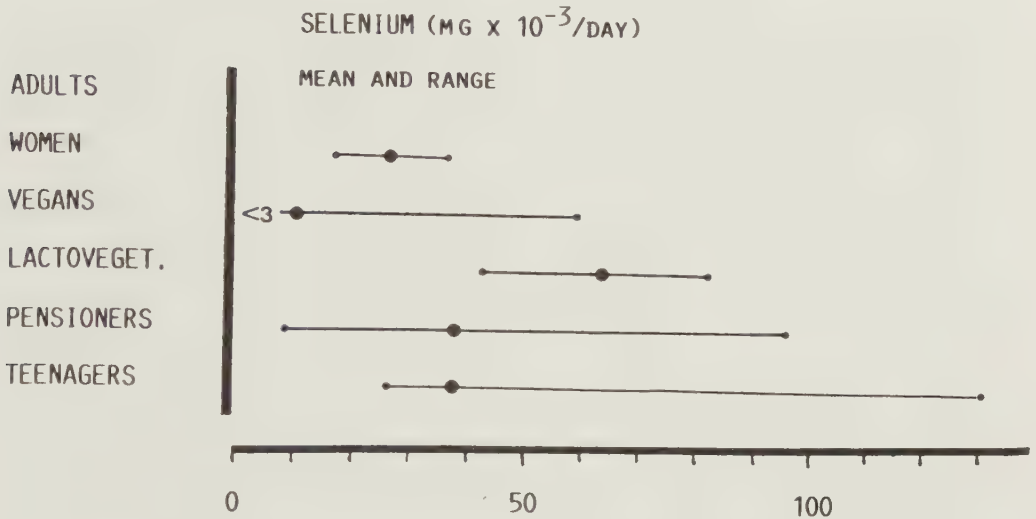
resemble manifestations of vitamin E deficiency and these abnormalities can be ameliorated by the dietary supplementation of sulphur-containing amino acids. The fact that selenium is an integral part of glutathione peroxidase in erythrocytes and other tissues partially explains the inter-relationship between the element and vitamin E. Moreover, selenium resembles sulphur in many aspects although it is not clear if selenium can replace sulphur in important metabolic reactions. Selenium may also have many other functions such as the maintenance of muscle integrity, involvement in nucleic acid metabolism, control of ion flux across the membranes, maintenance of sperm vigour and motility and participation in pancreatic functions (Frost and Lish 1975). In heavy metal poisoning, selenium has been found to reduce the toxicity considerably (Ganther et al 1972, Parizek et al 1971, Levander 1982, Hill 1975, Imura 1984, Ueda 1975). Recent epidemiological studies indicate that selenium deficiency may be associated with a number of diseases including cardiovascular diseases, cancer, multiple sclerosis and rheumatism (Wiliet 1985, Willet et al 1983, Schrauzer 1976, Schrauzer et al 1977, Westermarck 1977). In a very recent study, a selenium containing transfer ribonucleic acid has been isolated from mammalian cells, the significance of which is being investigated at the moment (Ching 1984).

From a geochemical and toxicological point of view of the known essential trace elements, selenium is the least abundant in nature and it is irregularly distributed in the soil. Because of this, selenium contents in food varies considerably from different geographic areas. Australia, New Zealand, certain parts of North America and Scandinavian countries are known to have considerably low contents of selenium in the soil. On the other hand, soil in some countries in South America, especially Venezuela is rich in selenium. In countries with low selenium levels in the soil, a minimum supplementary intake of selenium has been recommended for domestic animals (Underwood 1977). A working group within the Scandinavian Agricultural Research Association has recommended a minimum intake of $1.3 \mu\text{mol}$ (0.1 mg) selenium per kilogram fodder by the common domestic animals (Working Group 1975).

Selenium appears to be not essential for plants. Any selenium found in plants reflects the selenium contents in the soil. The capacity of plants to take up selenium from the soil depends upon the pH and presence of a number of other metals in the area where the plants are grown. Selenium contents of cereals may vary 1000-fold depending upon where the grain from which it is made is grown. On the other hand, selenium is essential for birds and animals and meat from animals meant for human consumption has always a certain amount of selenium. Selenium contents in milk, eggs and meat usually reflect the concentration of the element in plant and grain eaten by the animals and birds producing these foods. Supplementation of the soil and animal fodder with selenium is expected to increase the level of the element in the meat of the animals. The supplementation of animal fodder in Sweden, however, has not resulted in any significant increase of selenium in the meat and liver of pigs (Kolar 1983).

Figure X shows the intake of selenium in the diets analysed in the present study. The average content in the common diets is around 40 μmol (30 $\mu\text{g/day}$). The vegan diets had the lowest concentration of around 13 μmol (10 $\mu\text{g/day}$). The vegan diets consisted mainly of green vegetables grown locally. As mentioned earlier, since selenium is not essential for plants, the concentration of the element in vegetables reflect the selenium contents in the soil where it is grown. Like the soil in Finland and New Zealand, the Swedish soil is poor in selenium. The vegans also consumed nuts imported from other countries and a few 24-hour diets of the vegans had relatively high concentration of selenium. Such diets usually consisted of food materials imported from countries with high selenium in the soil. The lactovegetarian diets on the other hand had the highest amount of selenium when compared with the common and vegan diets (Abdulla and Dashti 1985b; Abdulla 1985c). The main reason for the increase of selenium in the lactovegetarian diets is probably due to the high percentage of imported food items in them. The lactovegetarian diets also consisted of a high proportion of milk and milk products. The diet of the teenagers had selenium levels similar to that of the other non-vegetarian groups.

Figure X: Concentration of selenium found in the diets analysed in the present investigation.



Selenium status is usually assessed by the concentration of the element in blood and the erythrocyte glutathione peroxidase activity. The blood concentration normally reflects the dietary intake (Stewart et al 1978, Schrauzer and White 1978). The body stores of selenium is also assessed from the concentration of the element in red blood cells, plasma, hair and nails. Erythrocyte selenium is considered to reflect the long-term store. The plasma-selenium on the other hand may indicate a short-term body store (Robinson 1982). Hair analysis is a good alternative when blood samples are difficult to obtain, e g infants and small children. There appears to be a good correlation between blood and hair selenium levels (Robinson 1982). Although the activity of glutathione peroxidase activity in erythrocytes has been used in the past as a measure of biological availability of selenium, there is growing awareness at present that there are other glutathione peroxidases which are not selenium dependent. Glutathione peroxidase activity in platelets may be a better indicator of selenium status than in erythrocytes (Bryant et al 1981, Kasperek et al 1979).

Only a limited number of blood analyses were performed in the group of subjects who participated in the present study. Selenium levels in the blood of subjects consuming common diets showed values ranging 38 - 96 μmol (30 - 76 μg)/l. A somewhat similar figure (71 - 102 μmol , 56 - 81 μg /l) has been found in the blood of apparently healthy Finnish adults (Levander 1982). A few blood samples from the vegans showed lower values (< 30 μmol , 24 μg /l) when compared with the levels found in other groups, but they did not manifest any overt signs or symptoms of selenium deficiency. Clinical follow-up of the pensioners for 14 years has also not shown any specific correlation between the low selenium levels in the diet and their general health. It appears that the population in Sweden has adapted to the low selenium contents in their diets. New Zealanders with low dietary intake levels of selenium similar to that of Sweden also have no overt signs of selenium deficiency (Robinson 1982, Robinson et al 1985). As in the case of other inorganic elements such as zinc and copper, subclinical deficiency which is difficult to detect at an early stage may occur in vulnerable groups in the general population. Only time alone can tell whether the general population living in countries with low selenium levels in the environment have an adequate selenium status or not.

Chromium

Chromium belongs to the Group VI A elements in the periodic table. It is present in all organic matter. Until a couple of decades ago, the principal interest of chromium in higher animals was confined to its toxic properties. Chromium exists in two valency forms and it is generally accepted that the hexavalent metal is much more toxic than the trivalent chromium. Only the trivalent chromium is biologically active. Prolonged exposure to chromium dust has been associated with an increased frequency of lung cancer (Brinton et al 1952). In sensitized individuals chromium exposure may induce allergic reactions.

The nutritional importance of chromium in animals was documented by Schwartz and Mertz in 1959. These authors showed that chromium deficiency impaired glucose tolerance in rats. Schroeder (1966, 1968) observed that chromium supplementation in rats and mice stimulated growth. Later studies by Mertz (1969) confirmed that chromium is one of the co-factors influencing the action of insulin. During the last two decades, several studies have been made to correlate carbohydrate metabolism, especially diabetes mellitus and chromium deficiency. It is generally accepted today that chromium in the form of naturally occurring dinicotinic acid-glutathione complex, named glucose tolerance factor (GTF) increase the effect of exogenous insulin on glucose metabolism. Oral administration of GTF to chromium deficient animals has been found to improve glucose tolerance (Mertz et al 1978, Doisy et al 1971). Studies in Turkey by Gurson and Saner have indicated that the administration of chromium compounds in children had beneficial effects on glucose tolerance and growth (Gurson and Saner 1973). Chromium may also effect lipid metabolism. Administration of chromium-rich yeast to healthy human adults result in a reduction of serum cholesterol (Doisy et al 1976, Liu et al 1977).

Chromium in minute concentrations is distributed in all human tissues. An adult human body is estimated to contain 100 - 200 μmol (5 - 10 mg) of chromium (Underwood 1977, Davidson et

al 1979). Tissue levels of chromium decreases with age. The concentration of chromium in the lung increases steadily throughout life. Chromium in blood is distributed partly in cells and partly in plasma. Plasma concentration is usually between 20 - 100 nmol (1 - 5 μg)/l. The mean concentration of chromium in erythrocytes is similar to that of plasma. The concentration of chromium in hair range from 3 to 6 μmol (150 - 300 μg)/kg and lower values may indicate deficiency of the element (Hambidge 1974).

Only little is known about the absorption of chromium in man. In animals, the ingested chromium is absorbed in the upper part of the small intestine (Donaldson and Berreras 1966). Inorganic trivalent chromium is poorly absorbed. Studies by Doisy et al (1968) indicated that only about 1 % of the orally administered chromium chloride is absorbed by human subjects. The absorbed metal is partly bound to transferrin and partly to GTF fraction (Doisy et al 1976, Hopkins and Schwarz 1964). Absorbed or injected chromium is excreted mainly via the urine. The daily urinary excretion range from 0.06 - 1 μmol (3 - 50 μg)/24 hours (Hambidge 1971, Wolf et al 1974). Urinary excretion in young healthy adults in the United States was found to be 0.19 μmol (10 μg)/day (Mertz et al 1978). Very small amounts of the absorbed chromium is lost via skin and bile. The unabsorbed chromium is excreted mainly in the feces.

In the present study, only the diets of the pensioners were analysed for chromium. The mean concentration in the pooled diets was 3.5 μmol (182 μg)/day. The female pensioners had a slightly lower intake than the males. The range for the whole material varied from 0.84 μmol (44 μg) to 11.3 μmol (588 μg). Contamination from stainless steel homogenizer, however, cannot be excluded completely. The dietary requirement of chromium in man is estimated from balance studies. According to Jeejeebhoy et al (1977) 0.39 μmol (20 μg) chromium/day is required for maintaining the balance. Insulin-requiring diabetic patients may need a higher amount of chromium for the same purpose. According to Mertz et al (1978) a safe intake is between 1 - 4 μmol (50 - 200 μg) per day. According to

Schroeder et al (1970) the average intake of chromium in the United States of America is $4.7 \mu\text{mol}$ ($245 \mu\text{g}$). Another study in USA showed a somewhat lower intake level (Levin et al 1968). Our results are very similar to the figures obtained by Schroeder.

Biologically active chromium is different in different food-stuffs. The highest chromium concentrations are found in nuts, spices and special herbs. Meat and vegetables are poor sources of chromium. Although we did not measure the plasma or urine levels of chromium in the pensioners who participated in the dietary survey, the clinical follow-up for 10 - 14 years did not show any overt signs of chromium deficiency related to carbohydrate metabolism. It is suggested that fasting blood glucose in subjects over 60 years of age should not exceed 5.6 mmol/l (Scherstén 1976). On the basis of this recommendation, we found that four pensioners had fasting blood glucose levels that were too high. The chromium concentration in the diet of these pensioners, however, were above $0.77 \mu\text{mol}$ (40 mg) which is twice more than the amount required for maintaining the balance. Our results on the whole indicated that the pensioners had adequate chromium intake.

Nickel

Nickel is an ubiquitous element in the biosphere. Nickel alloys are widely used for lining cooking vessels and in food industry. Traces of nickel are present in all animal and human tissues. Nickel deficiency has been documented in chick, cow, goat, sheep, minipig and rat (Nielsen 1982). The deficiency of this element in animals is associated with suboptimal liver function, abnormal hair characteristics, impaired reproductive functions and poor growth (Nielsen 1970, 1976). Completely convincing evidence that nickel is essential for man has not yet appeared. It is difficult to produce nickel deficiency in animals and there is no evidence of its existence in man. Elevated nickel concentration in plasma has been observed in patients who have suffered a myocardial infarction (Sunderman et al 1972). Like chromium, nickel produces allergic reactions in sensitized individuals

(Christiansen and Möller 1975). Oral ingestion of nickel in high doses can adversely effect copper and iron deficiencies (Nielsen 1982).

The exact physiological function of nickel in man is not known at present. Nickel is thought to act either as a co-factor or structural component of a number of enzymes, including arginase, urease, carboxylase and acetyl coenzyme A synthetase and trypsin (Nielsen and Ollerich 1974). Nickel is also present in RNA isolated from a number of sources and thought to play a part in maintaining the configuration of ribonuclease (King 1964). Nickel may facilitate the intestinal absorption of iron (Nielsen 1982). A nickel-containing macroglobulin, nickeloplasmin, has been isolated from human and rabbit serum (Sunderman et al 1972, Decsy and Sunderman 1972). The physiological function of this protein, however, is not very clear at present.

The exact amount of nickel present in an adult human body is not known at present. Nickel is considered to be an "ultra-trace element" and as such, by definition (Heydorn 1979) the total amount found in the body ought to be less than 80 - 160 μmol (5 - 10 mg). Most tissues of the human body do not accumulate nickel with age, except the lungs (Underwood 1977). Studies with labelled nickel in animals indicate that the element is distributed in small concentrations in all the tissues and the elimination is rapid. Injected radioactive nickel is eliminated completely within 48 hours. The nickel in blood is found partly in plasma and partly in the cells. The levels found in plasma is almost the same as that in erythrocytes. The concentration of nickel in plasma reported by various authors range from 0.3 - 0.9 μmol (20 - 60 μg)/l (Underwood 1977). The mean concentration of nickel in urine in a group of healthy US adults has been found to be 0.3 μmol (20 μg)/day (Sunderman 1965). Hair concentration of nickel in human females is significantly higher than that in the males. In one study, Schroeder and Nason (1969) found 0.07 mmol (4 mg) nickel per kg hair in females compared with 0.02 mmol (1 mg) in males. In this respect, nickel is similar to copper and cobalt.

The ingested nickel is poorly absorbed. The main route of the unabsorbed nickel is via feces. Some fecal nickel may come from bile (Onkelinx et al 1973). Generally speaking, only 5 - 10 % of the ingested nickel is absorbed (Linder 1978). Transport of nickel across the mucosal epithelium is an energy-driven process (Becker et al 1980). Nickel absorption may be affected by various dietary components, including iron (Solomons et al 1982).

Based on the data concerning the requirements of nickel in monogastric animals, Nielsen (1974) recommended an intake of $0.3 \mu\text{mol}$ ($16 \mu\text{g}$)/1 000 cal for man. In the present study, we have only analysed the nickel contents in the diet of the pensioners. We found a mean concentration of $7.3 \mu\text{mol}$ ($428 \mu\text{g}$)/day. The intake levels in the males and females were almost the same. Limited studies in other parts of the world indicate intake levels between 3 to $12 \mu\text{mol}$ ($170 - 700 \mu\text{g}$) per day (Nielsen 1982). Like chromium, nickel concentrations are very low in meat, milk and fruits. The highest concentrations are found in tea, coffee, cocoa, rice and vegetables. The vegetarian diets ought to be fairly rich in nickel. Cigarette smoking may accumulate nickel in the lung (Louria et al 1972). Drinking water in certain parts of the world, especially near nickel ores may contribute to high contents of nickel in the diets (McNeely et al 1972). As mentioned earlier, nickel alloys are widely used in food industry, especially in canning. Acidic fruit juices in cans may dissolve part of the nickel and may produce acute nickel toxicity symptoms in children. Fortunately, only a small part of the ingested nickel is absorbed. Toxic studies conducted in animals indicate that a concentration up to 17 mmol ($1\ 000 \text{ mg}$) per kg dietary food intake does not produce any signs of toxicity (Pathak and Pathwardhan 1952). The dietary intake of nickel found in the diet of the pensioners in Dalby is far below the toxic levels.

Iodine

The importance of iodine in human nutrition has been known to mankind since the early days of civilization. The use of iodine-containing medicaments for the treatment of goitre dates back into antiquity. The ancient Greeks, Chinese and Egyptians were reported to have used seaweed and burnt sponge which are rich in iodine for the treatment of goitre (Cavalieri 1973, Underwood 1977). Iodine was discovered by Courtois in 1811. The element was accidentally found by Courtois while manufacturing gun powder for Napoleon (Davidson et al 1979). In 1820, Coindet successfully treated goitre with tincture of iodine (Davidson et al 1979). The French Botanist, Chatin, later on carried out accurate and extensive analysis of iodine in foodstuffs and drinking water and documented that iodine was deficient in areas with endemic goitre (Underwood 1977). The first recorded therapeutic use of iodine was reported by the British Physician Prout in 1816 (Cavalieri 1973). The interest in iodine therapy for goitre was lost for a few decades after the disastrous attempt by Baussingault who administered toxic doses of iodine to school children in order to prevent goitre (Davidson et al 1979). Towards the end of the 19th century, it was discovered that the thyroid gland contained iodine and great interest was focused again on the role of iodine in human nutrition (Baumann 1896). Shortly after, Kendall and Osterberg (1919) in USA isolated thyroxin. Harrington and others continued the studies during the next few years and synthesized the thyroid hormones (Harrington and Barger 1927, Gross and Pitt-Rivers 1952). The first clinical experiment related to goitre and iodine therapy was documented by Marine and Kimball in 1920. The importance of adequate dietary iodine is now well-accepted by the public health authorities throughout the world.

All animals possessing thyroid tissue require iodine. Among the inorganic elements that have been found to be essential in human nutrition, iodine is unique in that it is an essential component of specific hormones. A healthy human body contains 160 - 400 μmol (20 - 50 mg) iodine of which the

major part (70 - 80 %) is found in the thyroid tissue (Davidson et al 1979). Certain tissues in the body other than thyroid, such as, salivary glands, gastric mucosa, lactating mammary glands and choroid plexus also possess the capacity to concentrate iodine, but they are not involved in the hormone synthesis. The level of iodine in the ovaries is 3 - 4 times that of muscle, but because of the large mass, muscle tissue as a whole, however, contains the next highest amount of iodine in the body after the thyroid. Significant concentration of iodine also occur in certain parts of the eye, especially the orbicular muscle (Underwood 1977)¹. Iodine found in the tissues and blood is partly in the organic form and partly as inorganic iodide. Inorganic iodine concentration found in the body are too low for direct measurements. In the thyroid gland, most of the iodine is in the organic form. The concentration of the inorganic iodine in the plasma, however, can be measured using radioactive iodine. The normal range of plasma inorganic iodine has been documented to be 0.6 - 5 nmol (80 - 600 ng)/l (Wayne et al 1964). The organic iodine found in the blood is mainly in the form of thyroxine bound to plasma proteins.

Since the only role of iodine is in the synthesis of thyroid hormones, the metabolism of iodine is closely linked to the thyroid function. For the details of the thyroid function, the reader is directed to any standard medical text book. The absorption of iodine in the food which is mainly in the inorganic form occurs throughout the gastrointestinal tract. The absorption is fast and complete. The renal excretion of iodine is through filtration and partial reabsorption. The amount of iodine that is excreted in the urine is proportional to the concentration in plasma. Only very small amounts of the dietary iodine is lost in the feces and as such, the urinary excretion of iodine indirectly reflects the intake.

The uptake of iodine in the thyroid is mediated through an "iodine pump" active transport mechanism which is inhibited by certain chemical agents such as perchlorate and thiocyanate. The iodine "trapped" in the thyroid gland is oxidised to iodine and coupled to the benzene ring of the tyrosine

molecule. In tissues outside the thyroid gland, the hormones are bound to thyroxin-binding globulins (TBG) and other plasma proteins. Small amounts of the hormone are taken up by the liver and excreted in the bile in the conjugated form. Part of this is reabsorbed and the rest is lost in the feces. Thus, most of the iodine in the thyroid hormones is used again after the metabolism of the hormones.

The average daily Western diet is estimated to contain 0.8 - 3 μmol (100 - 400 μg) of iodine, mainly in the form of iodide. Minimal requirements are in the region of 0.2 - 0.4 μmol (25 - 50 μg)/day and the optimal recommended intake is about 1.2 μmol (150 μg)/day (Laurell et al 1980). According to the Food and Nutritional Board (1970), the daily iodine requirement in adults is estimated to be 0.4 - 0.6 μmol (50 - 75 μg), or approximately 1 $\mu\text{g}/\text{kg}$ body-weight. Furthermore, an intake of 0.4 - 8.0 μmol (50 - 1 000 μg)/day by adults has been deemed to be safe. Many dietary components are poor in iodine and during the last few decades several measures have been taken to increase the dietary levels of iodine in Sweden and many other European countries. In 1966, the iodization of common salt was increased to 50 mg/kg, either in the form of potassium or sodium iodide. Apart from table salt and sea food, milk and dairy products are the most important sources of dietary iodine. During the early 1970's, the introduction of supplementation programme in cattle fodder increased the iodine contents of milk considerably (Iwarsson 1974). Significant amounts of iodine may also be introduced in the milk through the disinfection of the teats by iodine-containing agents (Iwarsson and Ekman 1974). A number of medicines, especially certain cough syrups may contain considerable amounts of iodine and this can increase the oral load to a great extent, especially in children. X-ray contrast material is another source of non-dietary iodine these days.

The iodine contents were analysed only in the diets of the pensioners and vegans who participated in the present study. The average intake of iodine in the diet of the pensioners was 2.2 μmol (282 μg)/day. When expressed as $\mu\text{g}/1\ 000\ \text{kCal}$

(4.2 MJ), the pensioners consumed 1.2 μmol (160 μg) iodine per day. The vegan diets on the other hand were very low in iodine. The average intake was only 0.6 μmol (70 μg) per day. When expressed as $\mu\text{g}/1\ 000\ \text{Kcal}$, it was only 0.23 μmol (29 μg) compared with the figure of 1.2 μmol (160) in the case of the pensioners. The vegans do not usually add any table salt in their diets. They also do not consume any dairy products. This may explain the very low contents of iodine in the vegan diets. According to RDA (1980), a daily intake of 0.7 μmol (90 μg) of iodine is considered to be adequate for women and 1 μmol (125 μg) for men in the age groups similar to that of the vegans included in the present study. The vegans did not, however, show any signs of thyroid deficiency.

The salt intake has been estimated in the dietary samples included in the present report. The average salt intake in the whole group (see section on sodium) is in the range of 4 - 8 g/day. If the salt is iodized, it should give about 1.5 μmol (200 μg) iodine from this source alone. Some information concerning the iodine contents in the diet also can be obtained from the intake of milk and other dairy products. Milk is estimated to provide 16 % of the dietary iodine. The average milk consumption in the general population in Sweden is around 400 ml/day and this should provide another 0.15 - 0.30 μmol (20 - 40 μg) of iodine per day. The consumption of herring and other fish which are rich in iodine is very common in the general population in Sweden and this may provide additional amounts of iodine in the diet. Thus, the average iodine contents in the common Swedish diets can be estimated to be around 2.4 - 3 μmol (300 - 400 μg)/day. Recently Gustavsson et al (1977), estimated the dietary intake of iodine and documented that the intake in Sweden has increased considerably during the last few years, mainly due to the overall use of iodized salt. Measurement of the thyroid hormones in the pensioners showed that they were close to the upper normal limit. Thus, it can be assumed that the

iodine intake at present in Sweden by the general population is more than adequate to maintain a normal thyroid function and in many cases, the intake may even be, unnecessarily large.

Manganese

Manganese has been known to be a constant component of plant and animal tissues since the early part of the present century. During the early 1930's it was shown that the element is essential for the growth of mice and rats (Kemmerer et al 1931, Orent and McCollum 1931). Later studies established that the element is essential for the growth, reproduction, skeletal development and brain functions of mammals and birds (Underwood 1977, Hurley 1982). In 1962, Leach and Muenster demonstrated that manganese played a very important role in the synthesis of mucopolysaccharides. Intensive research during the last few years has shown that manganese functions as a cofactor for a number of enzymes including glycosyl transferases (Leach and Lilburn 1978), pyruvate carboxylase (Utter 1976) and superoxide dismutase (McCord et al 1971). The manganese-containing superoxide dismutase is found in the mitochondria of the cells whereas the cytosolic superoxide dismutases contain copper and zinc. Manganese may also be involved in carbohydrate and lipid metabolism. The element has a lipotropic action in mice (Bell and Hurley 1973). In one male human volunteer, manganese deficiency resulted in hypocholesteremia (Doisy 1972). Manganese also plays a very prominent role in brain function (Hurley et al 1963, Papavasiliou 1981, Cotzias et al 1971).

An adult human body contains 0.2 - 0.4 mmol (10 - 20 mg) of manganese (Schroeder et al 1966). The skeleton and liver contain relatively high amounts of manganese. Tissues rich in mitochondria have a high content of manganese. The plasma level of manganese is around 20 - 30 nmol (1 - 1.5 µg)/l (Hurley 1982).

Only less than 5 % of the ingested manganese is absorbed and the absorbed element is mainly excreted via bile in the feces

(Underwood 1977, Britton and Cotzias 1966). Intestinal absorption of manganese may be affected by the presence of iron and phytates in the diet (King et al 1980, Gruden 1977, Davies and Nightingale 1975). Only very little manganese is excreted in the urine. In diseases of the kidney and during chelation therapy, the urinary excretion of manganese, however, may be increased.

Although experimental manganese deficiency has been reported in various animal species during the last couple of decades (Hurley 1982), there are no indications at present that manganese deficiency occurs in man. In 1972, Doisy reported a case of manganese deficiency in a male human volunteer who consumed a special diet deficient in the element for seven months. He developed abnormalities in the growth of hair and nail and suffered weight loss. He also had low cholesterol levels in the blood (Doisy 1972).

Estimations based on balance studies indicate that the human requirement is around 0.50 - 0.1 mmol (2.5 - 4 mg)/day (Hurley 1982). Human subjects who had manganese intake less than 0.04 mmol (2 mg)/day did not, however, show any adverse effects (Guthrie and Robinson 1977). The general recommendation for adults is around 0.04 mmol (2 mg)/day (Spencer et al 1979). Manganese content in foodstuffs of animal origin is very low whereas, vegetables, nuts and tea contains high amounts of the element. Tea is particularly rich in manganese (Wenlock et al 1979, Underwood 1977). Manganese has been analysed in a small number of diets investigated in the present study and found an average daily intake of 1.8 µg in nonvegetarians and 2.6 µg in vegetarians. The vegan diets have much higher concentration of manganese since they contain high contents of vegetables, nuts and tea.

Manganese toxicity has been reported in human subjects working in manganese mines and it produces neurological disorder similar to Parkinson's disease (Cotzias and Greenough 1958, Hurley 1982). From a human nutritional point of view, there is very little risk of manganese toxicity.

Arsenic, Cobalt, Molybdenum, Silicon, Tin and Vanadium

Rapid improvements in analytical technology and sophisticated instrumentation during the last few decades have enabled scientists to detect very small amounts of a number of inorganic chemical elements such as arsenic, cobalt, molybdenum, silicon, tin and vanadium in human tissues and circulating fluids. These elements are sometimes termed as "ultra-trace" micronutrients and by definition, their concentration in tissues is often less than 0.01 mg/kg (Heydorn 1979). That they are essential in human nutrition is not completely established for all these elements. In this report, only a short review of them will be given.

Arsenic

Arsenic, which is widely distributed in the biosphere, has been shown to be essential for the chick, goat, minipig and the rat (Nielsen 1982). It has been used by the ancient Greeks and Egyptians for the treatment of anemia and skin disorders. Complete evidence is lacking with regard to the absolute need for the element in man. No biological role or specific action of arsenic has been found so far. Arsenic may have a role related to the metabolism of phosphorous in biological systems (Benson et al 1980). The element also has been found to be associated with the activation of a few enzymes (Nielsen 1982). Recently, Nielsen has reported interaction of arsenic with zinc and arginine (Nielsen 1982).

The total amount of arsenic in an adult human body is less than 0.13 mmol (10 mg). Skin, nail and hair contain relatively high amounts of arsenic. Hair analysis is commonly used for the detection of arsenic poisoning.

The ingested arsenic, both inorganic and organic forms, is well absorbed from the gastrointestinal tract. The amount of arsenic absorbed, however, depend upon the chemical form of the element in the food. The organic form of the element which is found in high proportion in sea foods is absorbed to a greater degree than the inorganic arsenic. Arsenic ingested

in the inorganic form is also absorbed and partly methylated. The methylated as well as the inorganic forms are excreted in the urine. The unabsorbed arsenic is mainly excreted in the feces.

Most Western diets provide 0.25 - 1.7 μmol (20 - 130 μg) of arsenic (Jelinek and Corneliussen 1971). Diets high in fish and other sea foods provide much higher quantities of arsenic than diets rich in dairy products and vegetables. Until more is known about the physiological role of arsenic, it seems too early to judge the adequacy of the element in human diets. Although we did not analyse the arsenic contents of the population groups included in the present report, except a few dietary portions of the teen-agers, we can probably conclude that the concentration of the element is within the safe limits. In 15 dietary portions of the teen-agers, we found a mean arsenic level of 85 μg (Abdulla 1985d).

Cobalt

Cobalt has probably been known to mankind for centuries. Ceramics from 1500 B.C. have indicated traces of cobalt pigment. In 1926, Waltner and Waltner reported that large dose of cobalt-stimulated erythropoiesis in rats and induce polycythemia (Underwood 1977). The essential role of cobalt in animal nutrition came from the work of Underwood and others in 1935 (Underwood 1977). The discovery of vitamin B₁₂ ultimately confirmed the status of cobalt as an essential element in animal and human nutrition.

An adult human body contains 0.2 mmol (1.1 mg) of cobalt. The highest concentration is found in the liver, kidney and bones. Cobalt occurs in most of the tissues as vitamin B₁₂. A limited number of studies have documented the use of cobalt in the treatment of anemia, hypertension and goitre (Underwood 1977). The ingested cobalt from the diet is effectively absorbed and then most of it is excreted in the urine and feces. At the present time, there is no evidence for a dietary deficiency of cobalt in man. Most common diets in the Western countries contain much more cobalt than can be ac-

counted for as vitamin B₁₂. A typical Western diet provides 1.7 - 2.5 μmol (100 - 150 μg) per day. Green leafy vegetables are rich in cobalt.

Cobalt in large amounts induces polycythemia in experimental animals and man. The polycythemia in man induced by cobalt can be summated with the polycythemia of altitude and the marrow stimulation after bleeding (Underwood 1977). Cobalt in concentrations over 0.2 mmol (10 mg)/kg body weight in animals induce toxic symptoms, such as weight loss, anemia and death. A high dose of 5 mmol (300 mg)/kg body weight as soluble salt is lethal to sheep. Cobalt is used as an anti-foaming agent in the manufacture of beer and high consumption of beer containing toxic amounts of cobalt by humans has resulted in death due to heart failure (Louria et al 1972).

We did not analyse the cobalt contents in the diets of the various groups participating in the present study except a few vegan diets. In 10 dietary samples, the average level was found to be 110 μg . From the clinical follow-up of the pensioners, we have no reason to suspect any cobalt deficiency in the groups investigated.

Molybdenum

Molybdenum is a transitional element that is abundant in nature. Small concentrations of this element is found in all tissues of plants and animals. The essentiality of molybdenum for the growth of plants was established during the early 1930's (Underwood 1977). It was later found to be essential for all nitrogen-fixing organisms. Treatment with small amounts of molybdenum in molybdenum-deficient soil resulted in a high yield of crops. Certain plants such as blue lucera can selectively enrich the element and cattle ingesting such plants can suffer from molybdenum poisoning (Molybdenosis). Dietary ingestion of high copper can counteract the toxicity of molybdenum. Toxic effect of the element is more pronounced when there is a dietary deficiency of copper. Molybdenum has also been found to promote hepatic copper in experimental animals (Ferguson et al 1938). The copper/molybdenum inter-

action was later confirmed from studies done in Australia (Underwood 1977). Molybdenum also interacts with inorganic sulphate. In 1953, it was documented that xanthine oxidase is a molybdenum-containing enzyme (Richert and Westerfield 1953). Subsequently, molybdenum was shown to be essential for the growth of lambs, chicks and turkey (Underwood 1977). It has also been suggested that molybdenum may have a beneficial effect against dental caries (Davidson et al 1979). The importance of the element in human nutrition is not completely established at present.

Total molybdenum contents of an adult human body is estimated to be between 0.05 to 0.1 mmol (5 - 10 mg). The highest concentration is found in the liver, kidney, bone and skin. Only small amounts of the element is found in the blood. They range between 31 - 156 nmol (3 - 15 µg)/l. Bala and Liftshits (1966) reported a figure of 153 nmol (14.7 µg)/l of blood in healthy adults.

Molybdenum is rapidly absorbed from the gastrointestinal tract and transported to the liver. The main route of excretion of the element is via urine. Small amounts are excreted in the bile. Normal fecal losses amount to approximately one-third the urinary losses. Only limited data is available regarding the molybdenum contents in normal diets. A couple of studies indicate levels between 0.8 - 1.0 µmol (75 - 100 µg)/day (Underwood 1977). Dietary intake may, however, vary depending upon the geographic location, but is usually within the range 0.8 - 20 µmol (75 - 2 000 µg) per day. A very high incidence of gout in some parts of USSR has been attributed to a very high intake of molybdenum (Davidson et al 1979). A high molybdenum intake may also increase the urinary excretion of copper (Deosthale and Gopalan 1974). The retention of the element in the body is proportional to the protein contents in the diet. High dietary protein decreases the molybdenum retention whereas low levels of protein in the diet increase it's retention. Legumes, cereal grains, leafy vegetables, liver and kidney are good sources of molybdenum. High dietary contents of inorganic sulphate and tungsten inhibit the absorption and enhance the urinary excretion of

this element.

As mentioned earlier, there is little evidence regarding the essentiality of molybdenum in human nutrition. Recently, Abumrad et al (1981) described a case of possible molybdenum deficiency in a patient with short bowel syndrome receiving total parenteral nutrition. This patient had high urinary excretion of sulphate, xanthine, hypoxanthine and uric acid. These abnormalities indicated a deficiency of xanthine and sulphate oxidases. The clinical and biochemical abnormalities were corrected by the addition of ammonium molybdenate in the TPN solution. Estimated safe and adequate intake level is 1.5 - 5.0 μmol (150 - 500 μg)/day. Apart from the above-mentioned study, there is little evidence that molybdenum plays a significant role in any aspect of human health and disease at the moment. It is obvious that further studies are necessary before molybdenum attains the real status as an essential element in human nutrition. In the present report, the analysis of molybdenum is not included.

Silicon

In abundance in the biosphere, silicon is only second to oxygen. The contents of silicon in soil, plant and atmospheric dust is very high. During the early 1970's, Carlisle documented that silicon is essential for the growth of chicks (Carlisle 1970, 1974, 1976). Silicon deficiency in chicks and rats has been shown to be associated with abnormalities involving glycosamin glycans in the formation and structure of cartilage, bone matrix and connective tissue (Carlisle 1976, Schwarz and Milne 1972).

Significant amounts of silicon is found in tissues of animals and man. In adult human tissues, the concentration of silicon is around 1.8 - 36 mmol (50 - 1 000 mg)/kg (King and Belt 1938). In the human fetus, the lowest levels are found in the lung and highest in the muscles. In adult, the highest concentration is found in the lung. The high silicon contents in the lung of occupationally-exposed workers to silicon dust is associated with the development of malignant tumours of pleu-

ra and peritoneum. High concentration of silicon in the urinary tract is associated with urolithiasis, especially in animals (Underwood 1977).

Whether silicon is essential or not in human nutrition is not established. The element and its salt are poorly soluble in water and only little of the ingested element is absorbed. Diets of vegetable origin may contain relatively high amounts of silicon. No reliable data is available at present regarding the contents of silicon in human diets.

Tin

At present, there is no indication that tin fulfills the requirements for being an essential element in human nutrition. The element is irregularly distributed in nature. Schwarz in 1970 documented that tin stimulates the growth of rats (Schwarz 1977, Schwarz et al 1970). The sub-optimal growth in rats due to a possible deficiency of tin was related to riboflavin deficiency which in some not yet clearly understood way, associated with the oxidation-reduction potential of tin (Nielsen 1982). Tin has also been found to be a potential inducer of heme oxygenase in kidney (Kappas and Maines 1976). Small amounts of tin is found in human tissues such as teeth and lungs. There is no tin found in the human fetus.

Only little information is available at present regarding the contents of tin in prepared meals. Consumption of canned fruits and juices may contribute considerable amounts of tin in the food. The relatively high body burden of tin found in the adult body in affluent countries is thought to be due to the high consumption of canned foodstuffs. Average contents of tin in US diets has been found to be 17 - 34 μmol (2 - 4 mg)/day (Underwood 1977). Tin is poorly absorbed from the gastrointestinal tract and it is mainly excreted in the feces. Ingested tin has low toxicity, partly due to the poor absorption and retention. In general, orally administered tin as the inorganic salt has low toxicity in mammals (Schroeder et al 1964). From what has been known about the element so

far, it can be concluded that it is not an essential element in human nutrition. It is important, however, to consider tin from a toxicological point of view, in populations consuming large amounts of canned food-stuffs, especially in children.

Vanadium

Vanadium is known to be essential for plants and it is concentrated in the lipid fractions (Scanlon 1972). Conclusive evidence is lacking at present regarding the essentiality of this element in animal nutrition. Small quantities of this element is present in human tissues, especially in lung, bone, teeth and fat. The element can be accumulated in the lung mainly through inhalation. Blood contains approximately $0.2 - 0.4 \mu\text{mol}$ ($10 - 20 \mu\text{g}$)/l of vanadium (Allaway et al 1968). Söremark (1967) has, however, found somewhat lower values of vanadium in biological materials.

A well-balanced diet may provide $0.02 - 0.08 \text{ mmol}$ ($1 - 4 \text{ mg}$)/day (Davidson et al 1979, Czarnecki and Kritchevsky 1980) of vanadium. Radish is the richest source of vanadium. Diets rich in unsaturated fat (oil) contents may provide higher amounts of the element than diets rich in saturated fat. The ingested vanadium is poorly absorbed from the gastrointestinal tract. Inhaled vanadium accumulates slowly in the lung. Elimination of the ingested element is mainly through feces and urine.

Vanadium occurs in human enamel and teeth. Studies by Söremark and Ullberg indicated that the element may have some beneficial role against dental caries (Söremark and Ullberg 1962). Earlier studies on vanadium metabolism indicate that it may inhibit the synthesis of cholesterol. This action of vanadium is documented to be counteracted by manganese (Underwood 1977). Lewis in 1959 reported that occupationally exposed workers had lower cholesterol levels in their blood (Lewis 1959). Vanadium has also been found to inhibit a number of enzymes (Cantley et al 1977). Recent studies, on the other hand indicate that vanadium may act as a cofactor for certain enzymes (Nielsen 1982). An abnormal vanadium

metabolism has been suggested in a number of human disorders such as cardiovascular diseases, chronic renal diseases and kwashiorkor (Masiorni et al 1976, Bello-reuss et al 1979, Burger and Hogewind 1974). Although conclusive evidence is still lacking with regards to whether vanadium is truly essential in human nutrition, the dietary content of most diets consumed by the general population is more than sufficient to meet the possible requirements. Vanadium is not particularly toxic to man, except in case of very high occupational exposure. Intakes from 0.08 - 0.35 mmol (4 - 18 mg) per day in human subjects for 10 - 16 weeks have not produced any intolerance (Underwood 1977).

Lead, Cadmium and Mercury

Toxic metals, like lead, cadmium and mercury etc, have presumably always been constituents of man's environment. Metal poisoning as a human health hazard has been recognized for centuries. Mankind has used metals in a variety of ways throughout historical times and it is not surprising that metal poisoning has occurred now and then. Hippocrates in the year 370 B.C. described a case of abdominal colic associated with metal extraction. During the 10th century, Avicenna recognized for the first time cases of occupational mercury poisoning. In 1556, Agricola described toxic effects of arsenical cobalt in miners (Agricola 1556). Cadmium poisoning was described much later (Legge 1923).

In the past, human health problems from excessive exposure to heavy metals occurred under special environmental conditions. From the beginning of the current century, the implications of toxic metals in the general environment became a very important issue in many industrialized countries. Local and regional effects of chemical pollution were recognized with increasing knowledge of ecology. In countries with a high standard of living, metals are the basis of a multitude of materials and processes. There are continuous leakages of metals from mines, factories, industrial products, waste etc. The industrial use of potentially toxic metals has been growing steadily leading to continuous pollution of the en-

vironment (Tola 1973). Large scale mobilization and release of metals into the environment as a result of increased or altered energy techniques are potential problems whose magnitude has not been previously encountered (Fowler 1975, Sabadell and Axtmann 1975, Mac Gregor 1975).

Several tragedies of mankind during the past few decades are closely associated with accidental contamination of water and food by toxic metals. Infant mortality associated with lead poisoning from contamination of drinking water was very high in certain peat districts of England during the 1930's (Abdulla 1981). Itai-Itai disease due to cadmium poisoning and Minamata disease due to methyl mercury poisoning are other good examples illustrating the impact of metal contamination in the environment (Hagino 1959, Harada 1966). Barring occupational exposure, the major pathway through which the toxic metal enter the human body is via the food chain. In this report, a short summary of the dietary aspects of lead, cadmium and mercury will be presented.

Lead

Lead has been used in a variety of ways throughout historical times. The annual production of lead in the world at present is over 3 million tons. During the past few decades, about 5 - 10 % of the total production of lead was used in gasoline, a practice which is gradually diminishing at present. The lead used in gasoline is eventually discharged into the human environment. It may be inhaled or ingested through water and various foodstuffs, especially from green vegetables. Lead compounds are extensively used in the production of storage batteries and as stabilizers and pigments in plastics. Lead is also used in certain alloys and in crystal glass. Ship and car destruction plants represent another source of lead exposure. At high temperatures as in welding and cutting operations, lead is emitted from metal coated with lead-containing paints. Lead salts are also used frequently in the pottery industry. Food and beverages occasionally become contaminated by lead from glazes of earthenware used in the household. Food and beverages kept in soldered cans are another source

of lead exposure, especially if the material stored is acidic.

An adult body may contain 0.4 - 2.0 mmol (90 - 400 mg) of lead of which nearly 90 % is in the skeleton. Schroeder and Tipton found the mean total body burden of lead of 150 Americans to be 0.6 mmol (121 mg) (Schroeder and Tipton 1968). The concentration of the metal in tissues rises with increased exposure, particularly in the bone, liver and hair. The blood level of lead varies from country to country and average values around 80 μmol (165 μg)/l. Goldwater and Hoover reported a mean of 82 μmol (170 μg)/l for healthy humans with a range of 72 - 193 μmol (150 - 400 μg)/l (Goldwater and Hoover 1967). The values obtained from the blood of subjects living in the southern part of Sweden is around 48 μmol (100 μg)/l. The ingested lead is poorly absorbed and the major route of excretion is via feces. The normal absorption in a majority of cases has been found to be 5 - 10 %. A small amount of the absorbed lead is lost in urine, sweat and hair. In lactating women, small amounts of lead are also excreted in the milk.

As mentioned earlier, the major pathway through which lead enters the human body is via food and drinks. Cigarette smoking also may contribute significant amount of lead to the total body burden. Most dietary items normally contain only small amounts of lead except where the environment is contaminated with lead and food materials are cultivated in such areas. Only limited data is available at present regarding the contents of lead in prepared meals. Intake levels based on the expected levels of lead in food, air, water and from smoking in USA and Europe during the mid-1960's indicate approximately 1 - 2 μmol (200 - 400 μg)/day (Underwood 1977). In the present study, lead levels were measured only in the diet of the pensioners and 15 dietary portions of teen-agers. The average intake level for the male pensioners was 0.15 mol (30 μg) and for the female pensioners 0.09 μmol (19 μg) respectively per day. These figures are the lowest intake level reported until the present time. The results of another study on the lead contents in the diet of Swedish population

by Isacsson et al (1978), indicated an average intake level very close to the present findings. An intake level of $0.57 \mu\text{mol}$ ($113 \mu\text{g}$)/day has been reported from Finland (Schutz 1979). Jegrelius et al (1978), calculated the lead intake in Swedish diets on the basis of food consumption statistics and arrived at a figure of $0.39 \mu\text{mol}$ ($80 \mu\text{g}$)/day. From all these estimates, it appears that the dietary intake of lead in Swedish diets is low when compared with the reported values from other parts of Europe and USA. The low dietary intake found in the present study is in agreement with the low levels of lead found in the blood in the subjects who participated in the study. The average lead contents in the blood of the pensioners was $0.43 \mu\text{mol}$ ($88 \mu\text{g}$)/l (Schutz 1979). The average level of lead found in the 15 dietary portions of the teen-agers was $22 \mu\text{mol}$ ($44 \mu\text{g}$) (Abdulla 1985d). The dietary intake of lead can also be extrapolated from the data concerning the fecal excretion of lead. In one recent study, Piscator et al (1978), found an average daily excretion of $0.17 \mu\text{mol}$ ($35 \mu\text{g}$) lead in the feces of a group of normal healthy males in Sweden. Assuming a normal fecal excretion of 85 - 90 %, the intake levels ought to be around $0.19 - 0.22 \mu\text{mol}$ ($40 - 45 \mu\text{g}$)/day. The lower intake of lead found in Swedish diets may be due to a lower intake of canned foodstuffs by the general population. The environmental pollution in Sweden is also low when compared with that of more densely populated and heavily trafficked areas of Europe and USA. The provisional standards set by the FAO/WHO for tolerable intake of lead per week is $0.24 \mu\text{mol}$ ($50 \mu\text{g}$)/kg bodyweight and the present findings are below this (FAO/WHO 1972).

Consumption of illicit liquor and wine may contribute considerable amount of lead in the diet. Many of the common wines sold by the liquor shops in Sweden may contain as much as 5 - 6 μmol ($1.1 - 1.2 \text{ mg}$) lead/l. Thus, consumption of a single glass of wine may provide the same amount of lead as found in the rest of the diet (Karlsson and Sommarström 1978).

Infants and young children are particularly susceptible to the toxic effects of lead. A recent estimate from the United

States indicates that 600 000 children annually are shown to suffer from increased lead absorption (Anderson and Clark 1974). Many of these children may suffer from permanent neurological damage. Even a few deaths occur each year in the United States from lead-poisoning (National Academy of Sciences 1972). The major source of this type of exposure is from lead-based paints and lead-contaminated dust and soils in the urban and slum areas. Manifestations of lead poisoning are colic, encephalopathy, peripheral neuritis and anemia. For details of the hazards of lead exposure, the reader is directed to other literature published by the present author (Abdulla 1981).

Cadmium

Cadmium is uniformly distributed in the biosphere. Small quantities of the element has been observed in all living tissues. During the 1950's and 1960's, cadmium attracted considerable attention as an occupational hazard capable of causing pulmonary and renal dysfunction (Friberg 1950). Later reports from Japan indicated that cadmium could also be a serious problem from a public health point of view (Tsuchiya 1969, 1978). Excess of cadmium in the human body is not compatible with normal physiological functions. The metal is known to interfere with the metabolism of zinc and probably with other essential trace elements such as iron. The chemical and electronic structure of cadmium and zinc are very similar and it has been suggested that cadmium may perform certain physiological functions in mammals (Kägi and Vallee 1960). Conclusive evidence that cadmium is essential in human nutrition is still lacking.

The concentration of cadmium in an adult human body has been estimated to be 0.3 mmol (30 mg). The highest concentration is found in the kidney and liver. The kidney of the human fetus has almost no cadmium. The total cadmium in the human body accumulates until the 5th decade and then declines slightly (Underwood 1977, Davidson et al 1979). Blood of normal human subjects contain less than 89 nmol (10 µg)/l. Kubota et al reported a mean value of 62 nmol (7 µg)/l in the blood of 243 adults from 19 cities in the United States (Kubota et al 1968).

The ingested cadmium is poorly absorbed from the gastrointestinal tract. Approximately 5 % of the dietary cadmium is absorbed by humans (Flanagan et al 1978, Fox 1982). The unabsorbed cadmium is mainly excreted in the feces. Small amounts of the absorbed cadmium are excreted in the urine. The absorbed cadmium is accumulated in the kidneys and liver, bound primarily to metallothionein, a low molecular weight protein first isolated by Margoshes and Valle (1957). This protein can also bind zinc, copper and mercury. It is found in most tissues of the human body (Kägi, Nordberg 1979). When

there is no manifestation of renal damage due to cadmium exposure, urinary cadmium is a reasonably good index of the body burden (Friberg et al 1974, Elinder et al 1978).

Barring occupational exposure, the major pathway through which cadmium enters the human body is via the food chain and cigarette smoking. Recent advances in analytical technology has enabled the scientists to measure the contents of cadmium in food, blood, feces and urine very accurately. The intake levels reported from various countries in Europe and the United States indicate a figure of 0.13 - 0.26 μmol (15 - 30 μg) cadmium per day (Piscator 1982). In polluted areas, especially in certain parts of Japan, the daily intake may be 1.8 μmol (200 μg) or more (Tsuchiya and Iwao 1978). Renal dysfunctions, osteomalacia, pulmonary dysfunctions, anemia, slight liver damage and interference in the metabolism of essential elements such as calcium, zinc and iron are the major effects of cadmium poisoning.

The cadmium contents in the diets of the pensioners indicate an average intake of 104 nmol (11.7 μg)/day. This low figure obtained from the present study is similar to the findings by others in Sweden concerning the daily cadmium intake (Wester 1974, Kjellström et al 1978). An average level of 130 nmol (15 μg) cadmium was found in the diet of teen-agers (Abdulla 1985d). As in the case of lead, the dietary intake of cadmium can be calculated from the analysis of the metal in feces. A recent study by Kjellström et al indicate a fecal excretion of 116 nmol (13 μg) cadmium/day. This corresponds to an intake of 125 nmol (14 μg)/day (Kjellström et al 1978). This intake is very close to the findings reported in the present study. Smoking 20 cigarettes/day may contribute the same amount of cadmium to the body burden as in the daily diet (Piscator 1982). The tolerable weekly intake level of cadmium according to FAO/WHO is 63 - 71 nmol (7 - 8 μg) and the intake levels found in the present study is far below this limit. The low levels of cadmium in the diet is also reflected in the low level of the metal found in the blood of the pensioners (Abdulla 1985e).

Mercury

At present, there is no evidence suggesting an essential role of mercury in human nutrition. It occurs in very low concentrations in nature. Mankind has used the metal in a wide variety of ways since the days of Aristotle, who named it "quick silver". It has been used as a medicine in the treatment of venereal diseases during the Middle Ages. Since the beginning of the present century, the organic compounds of the metal has been widely used in agriculture as a fungicide. Mercury in nature is found in both inorganic and organic forms.

When metallic mercury is ingested, most of it is excreted in the feces. The absorption from the gastrointestinal tract is very low. Vapour from metallic mercury is more hazardous than the metal itself. This form of mercury poisoning occurs mainly in occupational settings. Almost 80 % of the inhaled vapour is retained. The biological half-life of the inhaled metal is around 50 days. A small amount of the inhaled vapour reaches the CNS and blood (Cherian et al 1978). The kidney takes up the major part of the inhaled metal. Prior consumption of ethyl alcohol is documented to reduce the uptake of the metal from the gastrointestinal tract (Hursh et al 1980). Acute exposure to metallic mercury can cause fever and pneumonitis, whereas chronic exposure results in CNS damage. Gingivitis may also occur in inorganic mercury poisoning. Long-term occupational exposure to inorganic mercury may also result in kidney damage (Buchet et al 1980). Inhaled mercury vapour crosses the placenta and may result in complications during pregnancy (Baranski and Szymczyk 1973). Inorganic mercury when used as a vermifuge has been documented to cause a peculiar disease in children which results in painful extremities (Swift 1914, Warkany and Hubbard 1953). Mercury salts, especially the mercuric chloride (sublimite) may be methylated in the intestine by the bacteria, especially the colonic flora and reabsorbed (Abdulla et al 1973a).

The organic mercury compounds, especially methyl mercury

are well-absorbed from the gastrointestinal tract. The major route of excretion of the ingested organic mercury is via feces and urine. The aryl and alkoxy compounds are the least toxic among the organo-mercury compounds. From a toxicological and public health point of view, methyl mercury is the most well-known compound. A number of catastrophic outbreaks of intoxication has been recorded. Japan experienced two episodes of methyl mercury poisoning during the 50's and 60's. Consumption of fish containing very high amounts of methyl mercury was the reason for these episodes in Japan. In other countries like Pakistan, Iraq and Guatemala, the methyl mercury poisoning originated from the consumption of bread and other wheat products which were treated with organo mercury fungicides. At the present time, the major pathway through which methyl mercury enters the human body is through the consumption of fish. Most of the mercury present in the fish is in the methylated form. Almost 90 % of the ingested methyl mercury is absorbed. After absorption, it is distributed to all tissues. As in the case of mercury vapour, methyl mercury crosses the blood-brain barrier and about 10 % of the absorbed amount reaches the CNS. Most of the methyl mercury found in the blood is in the red cells. Significant amounts of the mercury is accumulated in the hair. Analysis of the hair is widely used for the detection of methyl mercury poisoning (Wheatley 1979, Swedish Expert Group 1971). Methyl mercury has been documented to cross the placenta and brain damage may occur in infants (Amin-Zaki et al 1974). Methyl mercury poisoning in the adults mainly results in the damage of the CNS. After a latent period of 2 - 5 weeks from the exposure to methyl mercury, ataxia and visual disturbances are not uncommon. In severe cases of methyl mercury poisoning, paralysis and death may occur. In 1966, the use of alkylated mercury compounds in agriculture was banned in Sweden. During the last decade, it has been found that the methyl mercury content in fish caught in Swedish waters has decreased (Westöö and Öhlin 1975). Even the methyl mercury contents in the other foodstuffs have shown a constant decrease during the last few years (Berglund et al 1971, Kolar and Nilsson 1975, Westöö 1973).

The mercury content of a number of diets of the pensioners was determined and we found an average value of 18 nmol (3.6 µg)/day. Those dietary samples containing fish as the major component had almost twice the average contents of mercury. Dietary samples without any fish had as low as 10 nmol (2 µg) mercury/day. The mercury contents were also analysed in 147 diets of the normal healthy adults and we found an average value of 28 nmol (5.6 µg)/day. A number of these diets had no fish and the mercury contents were the same as that of the pensioners' diets (18 nmol; 3.6 µg). In another study by Wester, the average mercury content was 38 nmol (7.6 µg)/day which is fairly close to the figure obtained from the analysis of the diets of normal adults (Wester 1974). Preliminary results showing the intake of mercury in the diet of the teen-agers indicated values which were slightly higher than that were found in the diets of the elderly (Abdulla et al, 1985d). The present tolerable weekly intake of mercury set by FAO/WHO (1972) is 25 nmol (5 µg)/kg body weight. Our results showing the mercury content in the diets of the Swedish population are far below this limit.

Public health/Clinical Significance of Inorganic Chemical Elements

Rapid advances in analytical technology and sophisticated instrumentation during the last few years have certainly helped to bring about a breakthrough in the field of inorganic chemical element research. The importance of a number of these micronutrient elements in biochemical and physiologic processes is now well established at all levels of cellular complexity. Using the latest analytical techniques, researchers have documented the presence of more than 60 elements in bacteria, fungi, higher plants, animals and man. Trace elements, micronutrient elements or minor elements are terms applied to a number of these elements that are constantly found in biological systems in small concentrations. Complete evidence is lacking at present suggesting the essentiality of a number of inorganic elements that are found in living systems in very low concentrations (PPb levels).

The classification of the chemical elements from a nutritional point of view, however, presents a number of difficulties and at the present time, no classification is altogether satisfactory. The food that is generally consumed by the human race is derived from the sea or soil. This is applicable to both vegetarian and non-vegetarian diets. The chemical composition of the soil and sea depends on the rock that lie beneath them. It is obvious from this relationship that the concentration of inorganic chemical elements and their bioavailability depends on the geographic location from where the raw material for the diet is obtained. Salts of the electrolytes, sodium and potassium for example are usually water soluble and they are easily absorbed from the gastrointestinal tract. Salts of many other elements on the other hand are relatively insoluble and as such, the absorption of them from the alimentary canal is very poor.

In order to assess the nutritional importance of the inorganic chemical elements, it is relevant to consider the factors regulating their metabolism. The most important factors are:

- a) Bioavailability, solubility and permeability
- b) Transport
- c) The ultimate manifestation of a specific biological activity
- d) Storage and excretion

Once the essentiality of an inorganic chemical element has been established in human nutrition (Underwood 1977), the first important factor regulating its metabolism is the concentration and the chemical form in which it is found in the diet. From a public health point of view, it is important to assure the general population that the dietary intake of the element in question is adequate to meet the requirements. In spite of the impressive progress in inorganic chemical element nutrition during the last few decades, the minimum requirements of many of them are still hypothetical. It is generally believed that the minimum daily allowance (MDA) levels of many essential elements, including copper, chromium, manganese and selenium are so low that a pure nutrition-

al deficiency of these rarely occurs in man. On the other hand, the adequacy of a modern all-round Western diet as regards to inorganic chemical elements is presently being debated. Based on a limited number of balance studies, minimum requirements are available for a few essential elements such as zinc, copper and iron. If one accepts these figures, the intake levels of a number of inorganic elements in prepared meals will be found to be low in many affluent countries (Mertz 1970, 1981). Marginal deficiencies of several elements have been shown to exist in parts of the general population of industrialized countries (Mertz 1970). Whether we have adequate information or not concerning the minimum requirement for a particular chemical element, that has been found to be essential for human nutrition, the starting point, however, is to obtain reliable data concerning its concentration in prepared meals served on a plate for consumption. Such data cannot be obtained using conventional techniques such as diet history and interview studies. Direct analysis of the meals as they are consumed by the population can only give the true intake levels. The development and standardization of the duplicate portion technique has helped a great deal to solve this problem.

Once the exact dietary intake is known, it is necessary to know the bioavailability of the element in question. This depends on the chemical form of the element as well as the presence of other components in the diet. These matters are discussed to some extent in other sections of this report. Generally speaking, salts of the elements which are easily soluble in water and in the acidic environment of the stomach are more available for absorption than the insoluble ones. Once in the duodenum, the solution chemistry of the chemical elements determines their biological fate. Many of the elements are precipitated as insoluble compounds in the duodenum bound to molecules such as phytate, fiber, phosphate etc. These are thus made unavailable for absorption and excreted in the feces. It is thus, important to choose the right salt of the element when strategies for fortification and supplementation of diets with regard to inorganic chemical elements are planned. This is also important when normal feeding

through the mouth is not possible. Many elements coupled to certain amino acids and small organic molecules such as citrate are also absorbed effectively from the gastrointestinal tract. Once the inorganic elements are made available for absorption, they usually form complexes with endogenous chelating agents and are transported across the gut membrane. Certain elements which are soluble in the environment of the small intestine are transported into the enterocyte and then to the circulation by specific carriers. Specific transport proteins are known to exist only for a few elements. The principle function of the transport protein is to maintain the element in a soluble and assimilable form. A transport protein such as transferrin which normally carries iron also can transport zinc and perhaps other elements. Serum albumin is the most non-specific protein that carries a number of inorganic chemical elements. The amounts of these elements present in the tissue are regulated by the control of absorption in the alimentary canal, in which the enterocyte plays a very important role.

Except for a few elements such as iron, very little information is available regarding the ultimate biological activity of them. Although a number of them are assimilated in the liver, almost no evidence is available concerning the mechanism(s) by which they are incorporated into the macromolecules such as enzymes. Genetic factors are known to influence the assimilation of the chemical elements into the multiplicity of enzyme sites etc. A great deal of work is being done at the moment in order to clarify the physiologic activity, metabolic function and the exact loci of the inorganic chemical elements in living systems.

One of the most important problems concerned with inorganic chemical element nutrition is the diagnosis of deficiency. Severe deficiency that requires immediate medical attention occurs only rarely in affluent countries. Patients receiving long-term parenteral nutrition may develop acute deficiency symptoms of certain trace elements (Jeejeebhoy et al 1977). On the other hand, there are indications that marginal deficiencies of magnesium, copper, zinc, potassium and selenium,

are fairly common in parts of the populations of industrialized countries (Abdulla 1985c, Abdulla et al 1980b, 1982a and 1982b, Mertz 1981). The appearance of specific lesions or abnormalities due to the deficiency of a particular inorganic element is seldom obvious, especially if the deficiency is only mild. A lack of characteristic symptoms and diagnostic techniques is the major reason that these deficiencies are not detected at an early stage. Symptomatology and histopathology may vary depending upon a variety of factors such as sex, age and the duration of the deficiency. Even when the dietary intake is restricted, normal body functions are maintained for a certain period of time by homeostatic mechanisms and by making use of the body stores, if any. Mild deficiency of the elements may also be indistinguishable from primary deficiency due to starvation, loss of appetite or protein-calorie malnutrition. A number of the inorganic chemical elements have moreover, multiple mode of action in biological systems and a subclinical deficiency may not give rise to an abnormality in one specific biologic response. In addition, the marginal deficiency of certain elements such as zinc and copper may be made worse by the interaction of other metallic ions such as calcium. It is well known that a high intake of calcium can inhibit the absorption of iron, zinc, copper and magnesium. The calcium intake is relatively high in many affluent countries due to the increased consumption of milk and milk products and this may worsen the already low intake of elements such as magnesium, zinc and copper. Another problem associated with marginal deficiency of inorganic chemical elements is the selection of a diagnostic tool. Traditionally, plasma levels of the elements are measured to assess the body status. Plasma level of several trace elements, however, is a poor indicator of body status (Abdulla 1983a). The choice of a right specimen for analysis, which is truly representative of a metabolic event, may not be routinely available (Iyengar 1981, 1985). It is also important to consider the fact that correlation to disease states with concentrations of inorganic elements in tissues and body fluids may not necessarily give evidence for a cause and effect relationship. The biological action of many elements are often indirect. This can be illustrated by taking the

example of cobalt. The element alone is completely inefficient in the treatment of pernicious anemia. It is therapeutically significant only as a constituent of vitamin B₁₂ molecule. Ultimately, scientists will have to measure the concentration of the chemical elements at the sub-cellular, enzymatic and molecular levels to elucidate their role in diseases caused by marginal deficiencies. What can we do from a public health point of view until we have easy access to such techniques with regards to the detection of mild deficiencies of inorganic elements?

There are two approaches that have been suggested by experts to deal with the problem. The first one is to follow vulnerable groups in the general population, namely children, pregnant women, pensioners and alcoholics over extended periods and look for possible signs and symptoms of deficiencies. This approach, however, is expensive and time-consuming, but suited to study the long-term effects of marginal deficiencies. It may not be possible to carry out such studies in developing countries due to social and economic problems. In a country like Sweden, it is fairly easy to locate a vulnerable group such as the elderly with uniform living standards and eating habits. All the necessary statistics concerning their social and health status are readily available. In the present study, we have followed the health status of the pensioners for a period of 12 - 14 years. The elderly population on the other hand may not be the most ideal vulnerable group in the society to follow in order to detect the prevalence of marginal deficiency of inorganic chemical elements. With advancing age, a gradual deterioration of many physiological functions are to be expected. Energy requirements declines gradually during the last decades of life. Deficiencies in the dental status and digestive problems certainly influence the dietary habits of the elderly. Moreover, the senior citizens are often subjected to various drug therapies, especially diuretics, and thus may influence the metabolism of many chemical elements (Abdulla et al 1977).

Of the 37 Dalby pensioners clinically followed during the last 12 - 14 years, 25 are still alive today. From the clini-

cal evaluation which always included the laboratory data, we are unable to single out any sign or symptom that could be attributed to the low intake of the elements investigated. The plasma levels of most of the elements were within the normal reference range. It is very likely that the present RDA values are a little too high. It is also important to point out that for many inorganic elements, there are no internationally acceptable RDA values. Growing children and pregnant women are perhaps the most ideal group to follow in order to detect marginal deficiencies of inorganic chemical elements. These groups are known to be more susceptible to subclinical deficiencies of elements such as zinc, copper and iron than the rest of the population (Mertz 1970, Hambidge et al 1972, Jameson 1976, Abdulla and Dashti 1984b).

The second approach to identify the existence of marginal deficiencies of inorganic chemical elements rests in therapeutic trials. Many of the essential elements such as zinc, copper, iron and selenium are non-toxic in therapeutic doses. Studies in Egypt and a few countries in the Middle East on zinc deficiency and in China on selenium deficiency are good examples illustrating the positive effects of therapeutic trials (Prasad 1976, 1984, Chen et al 1980). Jameson documented the positive effect of oral zinc supplementation in pregnant women with low plasma zinc levels (Jameson 1976). Safe enrichment of table salt with iodine and iron and the therapeutic response of goitre and anemia are classical examples of supplementation studies. Recently, fortification of cow's milk with iron salts has been found to have a dramatic effect on iron deficiency anemia in Mexico (see chapter on iron). It might be worthwhile promoting similar strategies of fortification and supplementation to deal with deficiencies of other trace elements such as zinc, copper and selenium. In the past, more attention has been directed to studies in cattle than man to elucidate the health effects and interactions of inorganic chemical elements during supplementation programmes. It is perhaps more auspicious now to concern ourselves with human problems in this context. From a public health point of view, perhaps the time is ripe to do some intervention studies with regards to possible

trace element deficiencies. In developing countries, the authorities are more concerned about the more overt problems such as the protein-calory malnutrition and the trace element problems have a very low priority. Research done in affluent countries may, however, direct the attention of scientists and authorities to the equally important problems of trace element nutrition in the future.

CONCLUSIONS

Food tables documenting the concentration of various micro-nutrients in individual raw food materials intended for human consumption may not provide satisfactory information on the content of essential inorganic chemical elements, especially that of trace and ultra trace elements in prepared meals. Only direct chemical analysis of the actual food consumed during a 24-hour period can give an accurate estimate of the daily dietary intake of these elements. For investigating the dietary intakes of inorganic chemical elements in small, well-defined groups in the general population, the duplicate portion food sampling technique is a simple, practicable and reliable procedure. The validity of this technique can be verified by measuring the excretion of the electrolytes, sodium and potassium and nitrogen in 24-hour urine samples. Employing this technique, the concentration of sodium, potassium, calcium, magnesium, iron, zinc, copper and selenium in 882 daily dietary samples from various population groups in Sweden has been documented. In a limited number of samples, the concentrations of nickel, chromium, lead, cadmium and mercury have also been analysed. The results indicated that the concentrations of the elements, potassium, magnesium, zinc, copper and selenium in the majority of the diets were low when compared with the recommended dietary allowance (RDA) values. Vegetarian diets, however, had higher concentrations of these elements than the non-vegetarian diets. The relatively high concentration of raw food materials in the vegetarian diets may be one of the reasons for this difference. On the other hand, it is uncertain whether these elements in the vegetarian diets are biologically available. The concentration of the toxic elements, lead,

cadmium and mercury, in the diets analysed showed levels far below the permitted values. Chemical analysis gave lower values than are obtained by computation using food composition tables; their use therefore may not be ideal for estimating the dietary intake of trace and ultra-trace elements. At present, there are no suitable techniques for the early diagnosis of marginal deficiencies of trace elements. Plasma levels are poor indicators of body stores. A clinical follow-up study of the elderly included in the present study for a period of 10 - 12 years did not reveal any specific signs or symptoms of a deficiency of any trace element. In view of the fact that the intake levels of several inorganic chemical elements in the diets of the general population consuming common non-vegetarian diets are below the recommended levels, it is imperative to direct research to developing simple and sensitive techniques for the assessment of trace element status in vulnerable groups such as children, pregnant women, alcoholics and the elderly.

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Assessment of food consumption

CHAPTER 3

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For the assessment of food consumption, we have, in the present study, selected various components that are of special interest in the current debate and for which we have had laboratory facilities and techniques available. Total fat intake is, at present, regarded as being too high, and the fatty acid composition and the cholesterol content of foods have been under discussion with respect to their effects on our health status. Further, the carbohydrate composition of foods in our daily diet has several aspects of interest to contribute — one, for example, being the lactose content in subjects with lactase deficiency. Lactose intake is also an indicator of milk consumption and therefore reflects to some degree the calcium sources. Moreover, the "fibre hypothesis", in relation to several diseases, has had an impact on both nutritional advisors and on the general public at a stage in which the assembled information still manifests many uncertainties. In recent years, protein requirements have been re-evaluated in the light of the international debate. The new norms that have been promulgated signify that the protein resources can now meet the global needs, but only if adequately distributed. Additionally, protein requirements in old age seemed to us to be of special interest to study in a group that could be regarded as being relatively healthy. Likewise, vitamins and trace elements have evoked interest not only from the specific nutritional point of view for each subject, but also because they add information of

the adequacy of the food intake.

In Table 3.I, the components studied in the present investigations are listed. The duplicate portion technique offers special opportunities for the chemical analysis of toxic components as mercury, lead and cadmium, and also pesticides, nitrates and nitrite. It may represent a technique suitable for the continuous monitoring of factors in our environment that contaminate our foods.

Table 3.I. Components of the diet analysed by the duplicate portion technique

Energy	Calcium	Vitamin B ₁₂
Fat	Magnesium	Folate
Fatty acids	Total iron	Water
Sterols	Heme iron	Oxalic acid
Protein	Zinc	Nitrate
Amino acids	Copper	Nitrite
Carbohydrates	Chromium	Pesticides
Fibre	Nickel	Cadmium
Sodium	Selenium	Mercury
Potassium	Iodine	Lead

A. Methodological Considerations

Methods for the evaluation of food consumption from a qualitative and quantitative point of view have been in demand under very different perspectives. The fact that a high level of nutrition could be maintained in England dur-

ing World War II left the impression that after the war had ended there was no need for the support of either basic or applied research in nutrition (ARC/MRC *Food and Nutrition Research Report*, 1974). At present it is being realized that nutrition as an element in health presents problems that are more subtle and more difficult than, for example, those of manifest malnutrition as a cause of disease.

Food consumption can be measured by either indirect or direct assessment. The consumption of an entire population is indirectly estimated from food production statistics, and from imports and exports. Such methods are required for planning on a national basis. Obviously, only crude estimates can be obtained when related to the individual. Losses involved in the different steps of preparation and food processing may be difficult to identify. Figure 3.1 shows, for example, that in this Swedish community 24.2 per cent of the garbage originated from food products.

Information about the dietary intake and energy consumption of individuals can be obtained by different methods:

- The food is weighed by either the subject or the investigator before consumption and an equal portion is set aside for analysis.
- A record is kept by the subject of food eaten, which may be expressed in household measures, such as spoonfuls and slices, so that direct supervision by the investigator is not necessary.
- The subject recalls past dietary intakes and is interviewed in detail by the investigator.
- The duplicate portion technique, in which a copy of what is served and eaten is made up and used for chemical analysis.
- Determination of energy expenditure.

Each one of these methods for studying food consumption has its limitations. Methods based on the use of food tables require that such tables are up to date and complete to ensure that they represent the food currently eaten.

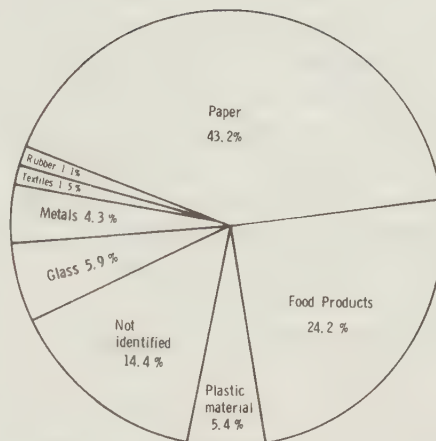


Figure 3.1. Analysis of the composition of garbage.

(Data kindly provided by the Avesta Community Council, Avesta, Sweden.)

Any method for the direct assessment of food intake requires considerable cooperation on the part of the subjects investigated. It is necessary to analyse, as far as possible, the characteristics of those studied, those not willing to participate as compared with the rest of the total population, as will be described in Chapter 5.

The accuracy of the information obtained depends on the subjects being surveyed. The aim is to get a record from a subject that is typical of that subject's usual consumption. Food consumption may vary over a given period of time. There can be considerable differences between what the same person eats in 2 different weeks (ARC/MRC *Food and Nutrition Research Report*, 1974) and on different days (Borgström et al., 1975). There can also be a variation between what the subject actually eats and what he reports that he has eaten or what an investigator observes that he has eaten. The survey itself may influence the food bought and consumed. With the dietary recall

method, there is no guarantee of the accuracy of the information provided by memory; this may be an increasing problem in old age. In our previous studies with the duplicate portion technique, in which some of us have taken part personally, we have experienced most of the difficulties emphasized by the ARC/MRC *Food and Nutrition Research Report* (1974). Some of them have been subjected to further examination, which will be described below.

1. Continuous or discontinuous sampling

As discussed earlier (Borgström et al., 1975), the duplicate food portions may be collected either on continuous days or with free days in between. The former approach has the advantage that the subject's intake for a longer period is studied, which should decrease the possibilities of intentional or unintentional changes in food habits due to the test situation. The intermittent approach, on the other hand, is less troublesome for the subject, with smaller risks for interruption of the sampling. It has been suggested that the two methods might give different results. To our knowledge, no precise scientific comparison of these two approaches is available.

An independent study in 22–26-year-old male medical students employing the duplicate portion technique with sampling on continuous days may therefore be used for comparison (Belfrage et al., 1973). This group had energy intakes very similar to those of 25–56-year-old men investigated by intermittent sampling (Borgström et al., 1975) as shown in Figure 3.2. Thus, no evidence was found that the method of sampling had a major influence on the measured energy intakes. For additional comparison, Figure 3.2 presents results from a study of 57-year-old men with peripheral vascular disease that were studied with the intermittent approach (Isacsson et al., 1978). The energy intake in this group was very similar to the energy intake of the male pensioners in the present report (group B in Figure 3.2). We have therefore felt justified to use the same technique – intermittent sampling – as in our previous series (Borgström et al., 1975).

2. Comparison between values obtained from food tables and chemical analysis of duplicate portions

To check the accuracy of food tables, a comparison was made between results from tables

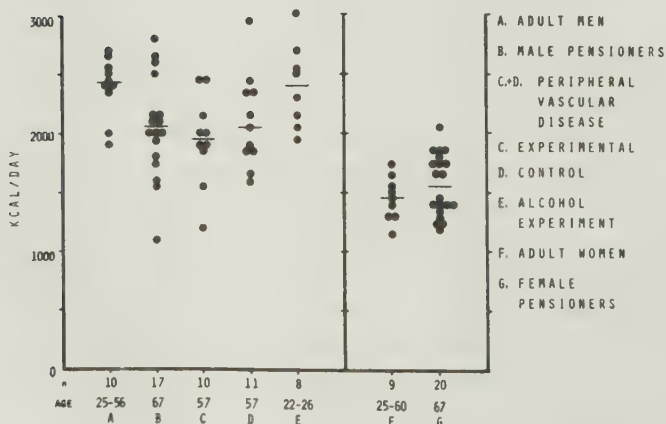


Figure 3.2. Energy intake in seven groups of subjects studied with the duplicate portion technique.

The data originated from the following sources:

- A, F – Borgström et al., 1975
- B, G – this report
- C, D – Isacsson et al., 1978
- E – Belfrage et al., 1973.

and from chemical analysis. Twenty diets were obtained from the hospital kitchen (University Hospital, Lund). Diets in the study were the normal hospital diet and a reduced protein diet. The compositions of the amounts of the different food items were known in detail and were used in the calculation of energy, fat, protein and carbohydrate by computer analysis, which was performed by AB Näringsdata (Malmö). Their data regarding the nutrient content of the food items were mainly obtained from Swedish food tables (Abramson, 1975), as well as directly from different food-producing industries. When no Swedish data were available, values from Danish, Norwegian or American food tables were used.

Methods that are described in the various chapters of the present report were employed for the chemical analysis of energy, fat, protein and carbohydrate. Dietary energy content was determined in two ways. First, by calculation from the chemically determined composition of fat, protein and carbohydrate multiplied by the respective energy factor (Chapter 8). Secondly, the energy content was determined by complete combustion of food samples to carbon dioxide and water in a bomb calorimeter (Gallenkamp). Corrections were made for the incomplete digestion of protein occurring in the human body, but not for the undigestible non-absorbable carbohydrate.

In Tables 3.II and 3.III the values obtained by computer and chemical analyses are shown for normal diets and protein reduced (40 g) diets, respectively. There was no significant difference between the mean values of any nutrient when comparing the two methods (t-test, $p > 0.1$ or non-parametric sign test). The correlation coefficients between the two methods were calculated using a non-parametric rank correlation test and were for energy (0.91^{xxx}), fat (0.97^{xxx}), protein (0.90^{xxx}), carbohydrate (0.86^{xxx}), calcium (0.71^{xx}) and iron (0.53^{xx}) ($xxx: 0.01 < p < 0.001$,

$xx: 0.05 < p < 0.01$). The coefficients indicated a high correlation.

As the chemical analyses and food table calculations are shown to be in close agreement, the main reason for quantitative differences between the duplicate portion method and interview techniques (dietary history, 24-hour recall, 7-day records, etc.) using food tables calculations is probably the different ways in which the material for analyses had been collected. In addition, components not determined by the present chemical techniques such as ethyl alcohol may lead to low values for energy.

3. Urinary analysis during food sampling and control periods

Do the participants continue their normal habits during the food sampling period? This aspect can be illustrated by a study of 60 women, 30–79 years of age, participating in a study of blood pressure in the Dalby population (Thulin, 1977). In the group of women the urinary volume, as well as the electrolyte excretions, has been investigated on three different occasions: once in 1972 and twice in 1975. The second time in 1975 each woman collected three urinary 24-hour portions and at the same time six daily portions of food by using the duplicate sampling portion technique. Urinary excretion studies were unfortunately not included in the present study in 1970–1971.

In Table 3.IV a comparison of the above-mentioned parameters has been made. No significant difference can be observed between the three examinations. When the older women, those above 55 years of age, were excluded from the group of 60 women, the same pattern was observed for them. Thus, we concluded that the food collection did not significantly disturb the dietary habits of these women. In addition, both the urinary volume and the elec-

Table 3.II. Normal hospital diet

	Energy (kcal)		bomb calori- metry	Fat (g)		Protein (g)		Carbohydrate (g)		Calcium (mg)		Iron (mg)		
	com- puter	chem- ical analysis		com- puter	chem- ical analysis	com- puter	chem- ical analysis	com- puter	chem- ical analysis	com- puter	chem- ical analysis	com- puter	chem- ical analysis	
Breakfast Standard ¹	182 906	165 815	176 846	3 34	1 27	6 37	6 38	31 110	— —	35 108	103 893	97 698	0.9 4.5	1.1 5.9
12/11 lunch dinner	513 239	522 190	546 192	15 6	9 5	26 32	33 23	64 13	64 7	74 11	59 237	115 151	5.2 5.3	3.3 2.0
13/11 lunch dinner	418 400	445 381	462 397	15 7	10 4	25 23	22 18	44 59	60 51	63 66	136 131	147 102	4.3 7.6	3.1 8.0
14/11 lunch dinner	505 405	651 418	672 429	7 21	20 21	46 24	45 34	60 27	60 18	70 21	1097 104	423 88	7.1 3.2	4.4 4.7
17/11 lunch dinner	462 366	473 399	496 408	4 18	4 16	34 17	34 19	71 33	60 40	72 42	310 78	227 127	5.3 3.5	4.3 2.8
18/11 lunch dinner	691 367	668 546	697 551	31 15	20 27	32 26	40 23	66 30	67 47	77 49	160 158	164 138	6.2 3.1	6.3 4.0
mean	437	462	485	14	14	28	29	47	47	55	247	168	5.1	4.3
S.D.	119	137	138	8	8	8	9	20	20	23	292	92	1.5	1.7

¹ Bread, butter, cheese, milk, fruit-juice: spread over all meals and meals in between.

Table 3.III. 40 g protein reduced diet

	Energy (kcal)			Fat (g)		Protein (g)		Carbohydrate (g)		Calcium (mg)		Iron (mg)	
	com-puter	chem-ical analysis	bomb calorimetry	com-puter	chem-ical analysis	com-puter	chem-ical analysis	com-puter	chem-ical analysis	com-puter	chem-ical analysis	com-puter	chem-ical analysis
Breakfast Standard ¹	99 596	89 522	91 541	4 37	3 31	7 5	7 9	9 64	9 61	137 92	83 82	0.9 4.4	0.9 4.5
3/11 lunch dinner	614 698	506 780	522 793	29 44	21 56	14 18	15 13	72 56	60 52	90 336	98 280	3.1 6.4	3.9 3.2
4/11 lunch dinner	698 758	656 693	675 715	40 44	35 36	15 14	18 17	65 72	63 71	189 119	107 88	4.7 3.2	3.0 3.5
5/11 lunch dinner	1004 631	880 451	900 460	66 40	58 29	17 12	16 12	78 50	69 33	200 111	185 81	3.3 2.7	2.0 1.9
6/11 lunch dinner	945 646	1063 606	1098 623	53 38	56 34	14 13	19 12	96 58	113 59	121 135	164 119	6.1 8.8	4.7 4.5
7/11 lunch dinner	609 956	527 937	545 965	30 60	22 58	16 14	16 14	64 85	62 83	102 180	126 131	3.9 5.8	3.1 3.0
mean	756	710	730	44	40	15	15	70	66	158	138	4.8	3.3
S.D.	146	202	197	12	15	2	3	13	21	70	57	1.8	1.8

¹Protein-free bread, butter, honey: spread over all meals and meals in between.

Table 3.IV. Urinary analyses of electrolytes during food sampling and control periods, in women participating in a blood pressure study (Thulin, 1977), see page 31

	1972 ¹	1975 I ¹	1975 II ²
A. 60 women, 30–79 years of age			
Number of urinary observations	60	60	180
Urinary volume (ml)	1166 ± 347	1266 ± 485	1152 ± 417
Sodium excretion (mmol/day)	126 ± 40	136 ± 54	120 ± 30
Potassium excretion (mmol/day)	63 ± 22	59 ± 19	57 ± 13
B. 26 women, 55–79 years of age			
Number of urinary observations	26	26	78
Urinary volume (ml)	1188 ± 294	1285 ± 479	1078 ± 241
Sodium excretion (mmol/day)	127 ± 42	133 ± 52	117 ± 28
Potassium excretion (mmol/day)	63 ± 23	54 ± 15	54 ± 12

¹One urinary 24-hour collection for each subject.

²Three urinary and six dietary 24-hour collections for each subject.

trolyte excretion values accord with a similar study in this country (Karlberg and Tolagen, 1977).

B. Practical Procedure

The days for food collection are given in Tables 3.V and 3.VI. Seven days have been used, each representing 1 day of the week. We have, however, generally tried to spread the sampling period over about 6 months rather than 7 weeks. This led to the loss of one male subject (060), who died between the two periods in 1971. In the female group, subject 054 only participated during one half, or 3 days, of the sampling period. These two subjects have therefore been excluded from the calculation of average intakes of specific components.

1. Instruction of the participants

Subjects selected for participation were contacted by the dietician. They were sent a letter of information about the study. Each relevant

household was then contacted and an appointment was made for a home visit. At the home visit the normal eating habits were discussed and the object of the nutritional study was explained with emphasis on the importance of not changing the normal dietary pattern. The technique for the duplicate portion sampling was explained. The day after the sampling had been completed the dietician returned and checked that no difficulties or deviations from the agreed procedure had occurred. She also checked the written diary, which had been made up during the day of study, with the participant.

Each participant received 150 Swedish kronor, which was estimated to cover the expenses for a guest in the household for 7 days, with the duplicate portion representing the guest.

2. Technique for the sampling of food

Everything consumed during the day had to be duplicated as exactly as possible by visual measurement. The duplicate food portion was

Table 3.V, 3.VI. Days studied. (The six-digit numbers refer to the year, month and day; Sunday–Wednesday in spring and Thursday–Saturday in the autumn.)

Table 3.V. Men

Subject no.	Su	M	Tu	W	Th	F	Sa
002	710502	710510	710518	710428	701008	700925	701003
005	710314	710315	710316	710317	700917	700911	700919
017	710502	710510	710518	710526	710513	710507	710522
026	710905	710913	710831	710908	700924	700911	700919
032	710509	710517	710427	710505	701008	700918	700905
036	710613	710614	710622	700616	700924	700911	700919
037	700607	700615	700609	700617	701119	701120	701121
038	710516	710426	710504	710512	700827	700904	700912
042	710425	710503	710511	710519	700716	700821	700829
044	700607	700601	700609	700603	701119	701120	701128
045	710509	710517	710427	710505	701008	700925	701003
056	700614	700629	700616	700701	701119	701120	701128
060					701008	700925	701003
067	700510	700525	700512	700429	701126	701127	701128
088	710509	710524	710511	710519	701001	700925	700926
090	710502	710510	710518	710526	700910	700828	700905
096	710516	710426	710504	710512	700924	701002	700912

Table 3.VI. Women

Subject no.	Su	M	Tu	W	Th	F	Sa
003	700607	700615	700609	700603	701119	701120	701128
008	710509	710517	710504	710526	701015	701009	701024
010	710613	710614	710622	710616	701008	701016	701003
013	710502	710510	710518	710428	700910	700918	700905
028	710425	710503	710511	710519	700827	700904	700912
034	710425	710503	710511	710519	700924	700911	700919
035	710425	710503	710511	710519	700827	700904	700912
039	710509	710517	710427	710505	700924	700911	700919
049	700510	700511	700519	700513	701119	701127	701128
054					700910	701002	700926
057	710516	710426	710504	710512	700827	700904	700912
064	700510	700511	700526	700520	701126	701120	701121
065	710502	710510	710518	710428	701008	701002	701010
069	700614	700608	700616	700610	701119	701120	701128
070	710425	710503	710511	710519	701001	700911	701003
076	710613	710614	710622	710616	700910	700828	700905
080	710425	710503	710511	710519	700716	700821	700829
081	710502	710510	710518	710428	700910	700828	700905
082	710620	710614	710622	710616	700827	700904	700912
084	700614	700608	700630	700610	701126	701127	701121

by and by put into a 2-litre Pure-Pak^{®1} container. The identity between the portion consumed and the duplicate was not checked by weighing, it being felt that such controls would make the procedure too complicated and would therefore interfere with normal habits.

Due care was taken to omit food not actually consumed. On the day of sampling, a written diary was made up including not only the type of food but also crude quantities, expressed in everyday language. The menus have been used for checking purposes and also for tracing the sources of unusual findings.

3. Extraction of food samples

The same procedure as previously described (Borgström et al., 1975) was used. After the duplicate portion had been collected, the container was stored in the icebox of the refrigerator until it was collected the following morning by the dietician for transport directly to the laboratory.

The contents were weighed and homogenized in a plastic beaker with an Ultra-Turrax stainless steel homogenizer. The homogenization time was usually 5 minutes, but this varied according to the composition and consistency of the food. To facilitate the emulsification of fat, 5 g of sodium desoxycholate dissolved in water was added. Whenever fat droplets became visible on the surface of the mixture or on the wall of the plastic beaker, the sample was heated to 40°C and homogenization was continued.

Exactly 100 ml of the homogenate was removed with a wide-mouthed pipette and transferred to a glass flask and lyophilized. The powder obtained by lyophilization was extracted twice with 200 and 100 ml of chloroform. During the extraction the mixture was

rapidly heated to 70°C. After filtration on a Büchner funnel, the lipid extract was washed with 50 ml methanol: water (1:1) and the mixture were left to stand overnight. The chloroform phase was dried with sodium sulphate and evaporated and the lipids were dissolved in 50 ml chloroform. The solution was stored at -20°C and used for analyses of fat-soluble components. The fat-free substance was recovered from the Büchner funnel and was dried under a vacuum over CaCl₂ in a desiccator overnight. After weighing, it was ground to fine powder and stored dry at +5°C.

Separate samples of the homogenate were collected for the determination of vitamin B₁₂, folate, DDT and other pesticides (Dencker et al., 1971), mercury and other metals (Dencker and Schütz, 1971).

The representativity and reproducibility of the lyophilization and excretion procedures were checked in the following way. Two aliquots of the homogenate were taken for analysis. One was immediately analysed, whereas the other was frozen and analysed later. The correlation between the dry weights of the fat-free and water-free substances obtained on two occasions was excellent (Figure 3.3). The corre-

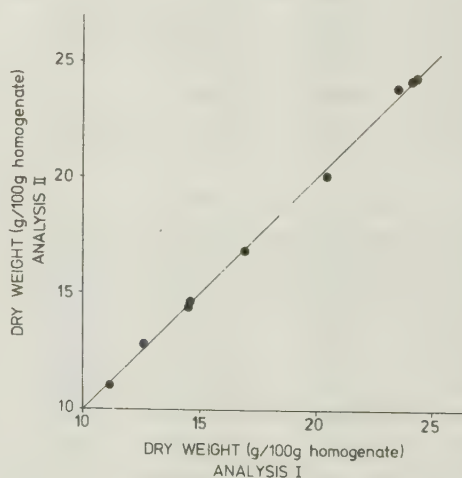


Figure 3.3. Correlation between dry weights obtained from 100 grams of homogenates on two occasions.

¹Pure-Pak[®], manufactured by Elopak on licence from Ex-Cell-O Corp.

lation coefficient was 0,999 and the variation coefficient, 0,81 per cent. The variation coefficient for the gravimetric determination of fat was 1,66 per cent.

C. Dietary Habits

In the individual subject, the most obvious source of information regarding food intake is from the case history. Simultaneous with the collection of the duplicate portion, a written diary was drawn up including the time and the type of food consumed. When the material had been assembled, the dietician went through the menu with the pensioner. Particular attention was paid to any deviations from the ordinary dietary habits.

Criteria for the composition of the meals have been developed by Carlgren and Rossander (1976). Seven different groups of food items that should preferably be represented in a well-balanced diet are recognized: vegetables; fruits and berries; potatoes and roots; milk and cheese; meat, fish and eggs; bread, flour and groats; and fat. Examples are given in Figures 3.4 and 3.5. As can be seen, the eating habits were retained, which is evident from the pattern during the autumn of 1970 and the spring of 1971.

Subject 010 has a very regular eating plan, whereas a more irregular plan for meals was illustrated by No. 026 (Figure 3.5), who during the 7 days had bread and butter in the morning at 6–7 a.m., sandwiches or an almost complete meal between 9 a.m. and 2 p.m. Only once – on Monday – did he have a full meal. He showed, however, the same pattern, during the autumn of 1970 and a year later.

The eating habits have also been illustrated for each individual as an average of the 7 days studied. Figure 3.6 gives the data for the 17 men and Figure 3.7 for the 20 women. At least one prepared meal was eaten each day. This is

further demonstrated in Table 3.VII. The most usual type of breakfast consisted of coffee and sandwiches. Two men and two women ate less than this for breakfast, whereas three men and two women had a more substantial breakfast. Twelve men and 14 women had a prepared meal at noon, but only seven men and seven women consumed a meal considered to meet optimal criteria according to Carlgren and Rossander (1976). Two men and two women had sandwiches during the day and dinner at night.

In no instance were any major differences recorded between the two periods used for the collection of the duplicate portions. This has been interpreted to mean that those studied probably retained their normal eating habits in spite of the investigation.

Table 3.VII.

	Types of meals	Women	Men
I	Breakfast plus one full meal	1	0
	Breakfast plus one full meal and one meal in between	0	0
	Breakfast plus one full meal and two meals in between	1	2
II	Breakfast plus one full meal and three meals in between	2	1
	Breakfast plus one full meal and four or five meals in between	1	2
III	Breakfast plus two full meals and one meal in between	6	2
	Breakfast plus two full meals and two meals in between	6	2
IV	Breakfast plus two full meals and three meals in between	3	6
	Breakfast plus two full meals and four or five meals in between	0	2

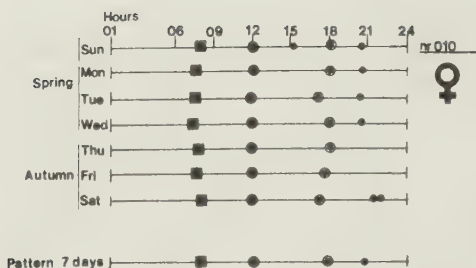


Figure 3.4. Dietary habits of subject no. 010.

- Prepared meal with five or more of the seven food groups represented
- ⊙ Prepared meal with less than five of the food groups, or sandwiches with four or more of the food groups represented
- Sandwiches with three of the food groups represented
- Coffee or tea with bread and butter, rolls, crumpets, or fruits
- ▲ Coffee or tea with or without biscuits, or sweets or snacks

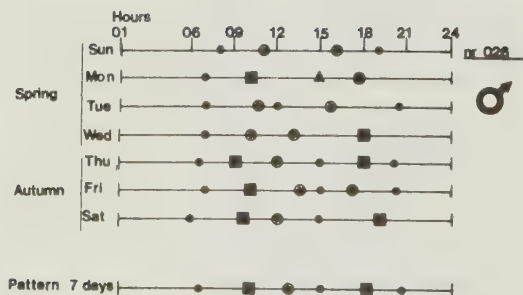


Figure 3.5. Dietary habits of subject no. 026.

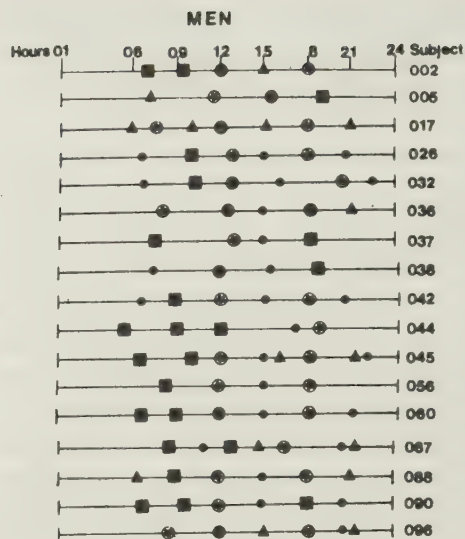


Figure 3.6. Dietary habits of 17 men - average pattern.

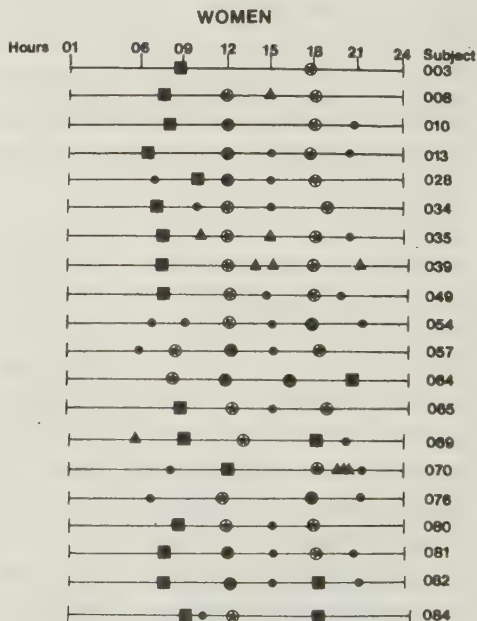


Figure 3.7. Dietary habits of 20 women - average pattern.

D. Intake per Day and Unit of Energy – 10 MJ or 1,000 kcal

In a round table discussion at the Second European Nutrition Conference in 1976 (Wretlind, 1977), *nutrient density*, or quality of a diet, was proposed as an alternative to absolute amounts for recommended dietary allowances. The *nutrient density* principle would ensure that essential nutrients are provided even for the low-energy consumer. The basis for this principle was outlined by Wretlind in 1967 and has been adopted by the Swedish Food Administration (*Statens Livsmedelsverks Författningssamling*, 1975).

A great variety of principles are being used in other countries. In the Soviet Union different allowances are made for people living in places with developed services and in places lacking services where the energy expenditure of the population is high. In all Eastern European countries the recommended protein allowances are higher than the values recommended by FAO/WHO experts; fat allowances are not specified; and vitamin C allowances are determined to "ensure optimal physical and mental efficiency" (Hejda, 1977).

The Swedish National Institute of Public Health published, in 1969, nutritional recommendations based on different age groups and sex: for infants 0–1 year, three categories; for children 1–10 years, six categories; for males 10–75 years, seven categories; etc. It shows a very complex system with subdivisions up to 55 years. In relation to old age, no differentiation is being made above 55 years for men and women. At the final discussion by the round table experts at the Second European Nutrition Conference, it was concluded that nutrient density, or nutrient concentration per energy unit, is a helpful way to express dietary recommendations. For evaluating diets or diagnosing inadequate intakes, however, the experts felt that it would be simpler to use

orthodox tables of nutrient requirements, expressed in age, sex and activity subgroups.

In the present report both the daily intake and the intake per 1,000 kcal, or 10 MJ, will therefore be given.

DISCUSSION

Assessment of food consumption is hampered by the lack of adequate methods suitable for normal daily living situations, as pointed out by the ARC/MRC *Food and Nutrition Research Report* (1974). The quantity of food needed requires insight into total energy needs; the quality of food necessitates analysis of the content of, for example, vitamins in the food and the corresponding vitamin status of the individual consumer. The latter question is in some respects, a simpler problem than the question of energy required. They are, however, both closely interrelated.

An intense debate on protein requirements in man started when it became evident that the present resources would not cover the needs of the world's growing population. In 1973, Durnin et al. therefore had cause to ask whether we know how much food man requires. Beaton (1975) pointed out the discrepancies in estimates of protein requirement based on balance studies and on studies of persons on a protein-free diet for the assessment of the need for protein compensation.

Garza et al. (1976) determined the effect of variations in energy intake on protein requirements. Individual energy requirements were based on weight changes and estimates of nitrogen balance. It was found that at the FAO/WHO safe level of egg protein (0.57 g/kg) an excess energy intake would be required to maintain nitrogen balance. Based on observations in four men aged 21–23 years with a body weight ranging from 55.9 kg to 80.9 kg and heights between 170 and 190 cm fed a for-

mula diet, it was suspected that a significant proportion of the population may require excess energy intakes to maintain nitrogen balance.

On the basis of animal studies, Thorbeck (1977) discussed the relation between energy intake and protein utilization. In animal nutrition an attempt is, for economical reasons, made to keep the intake of protein near the requirement. An intake of protein above the requirement for maximal protein gain in the growing animal will cause a higher O_2 consumption, thereby a higher heat production and lower efficiency of utilization of the energy intake (Thorbeck, 1977). Thorbeck (1977) emphasized the need to apply the experience from animal studies to man.

Only limited information is available concerning the needs of the group that the present studies are concerned with, namely, the ageing human being. Dietary studies in old people (covering 1 week) including a clinical examination and an analysis of blood samples were conducted by the British Department of Health and Social Security (1972) in 879 men and women in six different areas. The subjects were selected from the registers of the local executive councils of the National Health Service and formed a representative sample of all aged people in the country. The results showed that 3.2 per cent of those over 65 years of age were malnourished. In the 27 subjects found to be malnourished, two (0.2 per cent) had frank scurvy and eight (0.9 per cent) angular stomatitis. The reason given for the diagnosis was in most cases extreme emaciation. In 12 of the subjects there were major medical disorders that may have contributed to the malnutrition.

Because of the findings of scurvy in the British study of old people, Brante and Jägerstad (unpublished) have analysed the ascorbic acid content of whole blood in Dalby pensioners born in 1903. Median values for 37

men were 0.057 mmol per litre (range 0.017–0.085 mmol per litre); 12 men had values below 0.034 mmol per litre, which has been suggested as the lower normal limit. In 25 women median values were 0.074 mmol per litre (range 0.023–0.125 mmol per litre). Five women had values lower than 0.034 mmol per litre. These observations suggest the need for further studies of the ascorbic acid status in old people. This was not planned when the present studies were conducted in 1970.

In the very large survey "Nutrition Canada" no clear clinical evidence of vitamin C deficiency was observed in the general population. Mild to moderate vitamin C deficiency was indicated in a small number of young Indian men. Eskimos had clinical signs of vitamin C deficiency. Intakes below adequate levels occurred in 90 per cent of senior adult Eskimos.

The duplicate portion technique has so far only been used to a limited extent for the assessment of food consumption, as reviewed by Borgström et al (1975). A study with 1-week sampling and chemical analysis was recently described by Riis and Pedersen (1977). This investigation comprised 20 men aged 40–49 years living in Oslo, Norway. The mean dietary energy intake was 2.215 kcal (9.2 MJ), with a standard deviation of 573 kcal (2.4 MJ). The mean fat intake was $91 \text{ g} \pm 27 \text{ g}$ and the food nitrogen content $11.5 \text{ g} \pm 2.4 \text{ g}$. The mean distribution of energy from nutrients was fats 37 per cent, proteins 13 per cent, carbohydrates 40.5 per cent, alcohol 9.5 per cent. The mean cholesterol intake was $313 \pm 96 \text{ mg}$ and the sum of cholesterol, β -sitosterol and campesterol in the diets was $412 \pm 107 \text{ mg}$. The authors observed that the results were well below those expected.

In our previous study (Borgström et al., 1975) in 10 men aged 25–60 years, the median values for energy were 2.425 kcal (10.1 MJ) when the carbohydrate was estimated by difference and 2.225 kcal (9.3 MJ) when chemi-

cally determined. In the Norwegian study, 9.5 per cent of the energy originated from alcohol – the median value in our study was 60 kcal (0–230), or 2.5 per cent. The median cholesterol intake per 1,000 kcal was in the Norwegian study 139 mg and in the Swedish 96 mg. Riis and Pedersen (1977) concluded that the reliability of the duplicate portion technique must be questioned.

The International Agency for Research on Cancer Intestinal Microecology in a study on dietary fibre (1977) collected food by the duplicate portion technique for analysis on a Monday, the fourth and last day in a 4-day period during which food diaries were kept. A comparison was made between 55–64-year-old

males in Copenhagen, Denmark and Kuopio, Finland. The mean ($\pm$ S.D.) intakes were in Copenhagen fat 79.2 ± 25.2 g, protein 52.6 ± 15.3 g, and energy 7.9 ± 1.9 MJ. In Kuopio the corresponding figures were fat 91.4 ± 28.9 g, protein 52.6 ± 15.3 g and energy 10.3 ± 2.4 MJ.

It seems to us that the duplicate portion technique gives results that in different series are on the whole similar. It provides an instrument for measurements of ingredients that have not been possible to determine up to now. The increasing interest in tracing carcinogenic components in food can only be met by techniques that make use of the material actually consumed.

Sodium

CHAPTER 13

SODIUM

*MOHAMMED ABDULLA, MARGARETHA JÄGERSTAD, ÅKE NORDÉN, THOMAS THULIN
and SVEN SVENSSON*

CHAPTER 13

SODIUM

*MOHAMMED ABDULLA, MARGARETHA JÄGERSTAD, ÅKE NORDÉN, THOMAS THULIN
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The addition of salt to food is a dietary practice as ancient as the earliest known civilizations. There is, however, some historic evidence that ancient Egyptian philosophers and Indian monks avoided excess salt intake. Egyptian priests considered salt to be an inducer of depression, and ancient Indians avoided salt to counteract the poisonous effects of snake-bites. Although superficial, it is interesting to note that from the very early days of human history excess salt was associated with a tinge of suspicion or fear. It is also known today that generations of various tribes in Africa and Asia survived without the addition of salt to their foods. On a global basis, a lower dietary intake of salt was a common factor in all primitive civilizations. Today, considerable quantities of salt are added to foods in most societies.

From a physiological point of view, a balance between intracellular potassium and extracellular sodium is of great importance to the normal functions of the body. The concentrations are under homeostatic control and are regulated by the kidneys and influenced by adrenocortical steroids. Kidney diseases, as well as gastro-intestinal disorders, can disturb the electrolyte balance. In elderly persons, both of these conditions are common. Certain drugs, especially the diuretics, which are often taken by the elderly, can easily upset the balance of electrolytes (Laurence, 1973).

It has been proposed that chronic, excess consumption of salt is involved in the pathogen-

esis of hypertension in man (Dahl and Love, 1954; Dahl, 1961, 1972; Meneely and Dahl, 1961, 1972; Freis, 1976), and a large reduction in dietary salt intake can result in a lowering of blood pressure in some people (Corcoran et al., 1951; Dole et al., 1951).

Animal experiments have shown that an excessive intake of salt over a long period of time is accompanied by an increased blood pressure (Ambard et al., 1904; Koletsky, 1961; Meneely et al., 1953; Knudsen et al., 1970). With this background, we have investigated the dietary intake of salt in the pensioner group at Dalby.

MATERIAL AND METHODS

The collection and handling of the dietary portions are described in Chapter 3. A total of 115 portions from 17 men and 136 daily portions from 20 women were analysed. All chemicals used were of analytical grade. The glassware and pumice stones were soaked in 50 per cent concentrated nitric acid and rinsed several times in ion-free water (deionized and glass-bi-distilled) prior to each analysis.

The wet-ashing was performed as follows: 0.5 g of the food-powder was suspended in 2 ml ion-free water in a 20 ml Kjeldahl flask. To this, 0.5 ml concentrated sulphuric acid, 0.5 ml concentrated nitric acid and a few pumice stones were added. The flasks were placed on a thermostatically controlled sand bath. The mix-

tures were allowed to decompose until most of the nitrogenous gases had disappeared. The addition of concentrated nitric acid, 0.250 ml at a time, was continued until the organic material was completely digested. Normally, five to eight aliquots of concentrated nitric acid are required.

At this stage the flasks were removed from the sand bath and allowed to cool. As a final stage of the digestion, 250 μ l of a mixture containing equal amounts of concentrated perchloric acid and concentrated nitric acid was added. After the addition of this final mixture, the flasks were allowed to stand on the sand bath until the solution was colourless. At this stage only the concentrated sulphuric acid was left in the flask. Normally, the whole procedure takes 6–8 hours and during the first 4 hours careful observation is necessary due to the initial vigorous reaction.

Blank samples were prepared on each occasion of wet-ashing by using the same amounts of concentrated acids. The final volume of concentrated sulphuric acid after the complete digestion was diluted to 10 ml with ion-free water after cooling.

The sodium was determined with a flame photometer (Instrument Lab., Inc. Model 343). In order to see if fat extraction with organic solvents and the lyophilization procedure resulted in any loss of sodium, we determined the sodium contents in 10 freshly homogenized daily portions as well as in the corresponding lyophilized powder. The homogenized portions contained 10 per cent more sodium than the lyophilized material. We have not made any correction for this loss in the final calculations. Further, the high temperature produced during wet-ashing (250°C) may have resulted in additional losses of sodium. We have also tried to make a direct extraction of sodium by shaking a known amount of the lyophilized powder with a 2 per cent solution of nitric acid. In spite of the fact that the organic bound sodium

cannot be extracted by simple shaking, we obtained more than 90 per cent sodium as compared with the results obtained from the wet-ashing of the lyophilized powder. By a combination of direct shaking and wet-hydrolysis, we have estimated the losses due to high temperature to be less than 5 per cent.

Since we had not originally planned to analyse sodium in the food samples, 5 g of sodium desoxycholate was added as an emulsifier in each daily portion during homogenization, and consequently this will result in an additional amount of sodium in the final calculation. It has been calculated to be 10 mmol per portion. No corrections for this addition have been made in the tables because the losses during analysis of sodium were estimated to be of the same magnitude, namely 10 mmol.

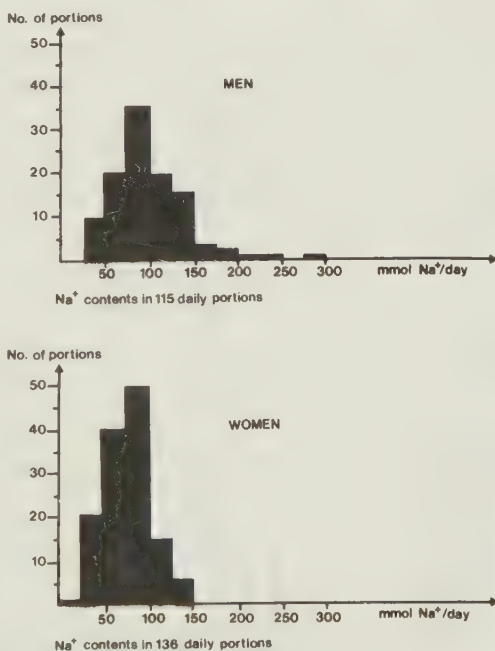


Figure 13.1. The median daily intake of sodium expressed in mmol among 16 male and 19 female pensioners.

RESULTS

In Figure 13.1 and Tables 13.I and 13.II, the complete results are shown. The median daily intake in the male pensioners was 88 mmol, with a range of 65–151 mmol. Corresponding values for the female pensioners were 77 mmol (44–106 mmol). The distribution of the sodium content of the individual diets is shown in Figure 13.2. The lowest sodium content among the men was 30 mmol and the highest 237 mmol and for the women 24 and 173 mmol, respectively.

DISCUSSION

Most people in the industrialized countries consume 80–300 mmol of sodium daily (4.7–17 g sodium chloride) (*Recommended Dietary Allowances*, 1974). The major source of sodium in common foods is the salt used in cooking and seasoning. Industrial processing of the food material and the nutritional additives, which normally contain sodium salts, are some factors influencing the dietary intake of sodium. In addition to salted foods, many of the

common food items, such as bread, cheese, milk, eggs, etc., contain large amounts of sodium. Snack foods like crisps (potato chips) and nuts, which are very common these days, also contain considerable amounts of salt.

Most of the daily output of sodium is in the urine, which normally contains 100–150 mmol per 24 hours. By way of perspiration, a considerable loss of sodium can occur. Normal sweating results in the loss of 20–50 mmol sodium daily. Small amounts of sodium (less than 5 mmol per day) are also lost in the faeces. Heavy exercise, environmental heat and high fever are some other factors that influence sodium loss.

Although we have no data regarding the daily output of sodium in the pensioners, we have studied the relation between the dietary intake and 24 hour urinary output of sodium in a group of women in Dalby using the same technique. The results indicated that the intake was slightly less than the urinary output. When we made the necessary corrections for the losses prior to analysis, there was a good correlation between the intake and output (see Chapter 3).

The importance of salt in the pathogenesis



Figure 13.2. The distribution of the sodium content in the individual diets.

Table 13.I. Sodium intake in the male pensioners (mmol/day)

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
002	123	147	140	124	97	212	139	139	97–212
005	87	98	52	37	84	114	85	85	37–114
017	74	77	116	144	237	99	81	99	74–237
026	39	54	69	75	73	72	30	69	30– 75
032	133	101	116	78	106	75	140	106	75–140
036	55	79	87	52	65	40	66	65	40– 87
037	107	87	48	117	76	89	65	87	48–117
038	61	42	75	51	44	103	63	61	42–103
042	129	83	174	101	84	82	71	84	71–174
044	83	91	83	100	88	106	91	91	83–106
045	118	179	128	127	149	118	104	127	104–179
056	79	71	77	83	39	81	49	77	39– 81
060 ¹	–	–	–	192	182	117	–	–	–
067	88	71	128	70	57	83	116	83	57–128
088	138	88	151	151	195	294	145	151	88–294
090	82	76	62	51	97	89	100	82	51–100
096	153	95	110	139	137	99	148	139	95–153
Mean	100 mmol		Median	88 mmol (44 mmol/1,000 kcal)					
S.D.	43 mmol		Range	61–151 mmol					
									n = 115 daily food portions

Table 13.II. Sodium intake in the female pensioners (mmol/day)

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
003	68	50	31	76	56	123	84	68	31–123
008	98	71	114	127	88	100	94	98	71–127
010	85	87	80	59	83	83	92	83	59– 92
013	148	97	99	106	111	97	120	106	97–148
028	69	61	58	41	50	42	40	50	40– 69
034	86	89	73	54	110	63	91	86	54–110
035	97	99	76	80	88	81	59	81	59– 99
039	71	122	76	79	88	131	92	88	71–131
049	97	83	73	79	84	63	83	83	63– 97
054 ¹	–	–	–	173	131	90	–	–	90–173
057	59	91	73	45	88	36	63	63	36– 91
064	121	82	70	58	90	61	92	82	58–121
065	78	72	136	85	76	72	71	76	71–136
069	44	33	117	53	79	52	44	52	33–117
070	44	44	34	48	48	58	37	44	34– 58
076	38	82	41	95	53	51	24	51	24– 95
080	63	121	117	91	69	78	61	78	61–121
081	42	36	58	45	67	40	57	45	36– 67
082	61	55	97	103	85	101	51	85	51–103
084	108	133	129	55	66	81	91	91	55–129
Mean	78 mmol		Median	77 mmol (47 mmol/1,000 kcal)					
S.D.	27 mmol		Range	44–106 mmol					
									n = 136 daily food portions

¹ Excluded from the calculation of the mean, S.D., median and range for the whole group.

of hypertension was emphasized as early as 1904 by Ambard and Beaujard (Ambard et al., 1904). Epidemiological studies have shown (Freis, 1976) that when salt is not added to the diet the prevalence of hypertension is low or absent, and when salt is used the prevalence of hypertension is high. An absence of hypertension and a failure of the blood pressure to rise with age has been observed in unacculturated societies from widely different parts of the world. A number of studies suggest that it is the lack of salt in the diet that accounts for the virtual absence of hypertension. When these people, who are free of hypertension, adopt modern ways of life, blood pressure rises and hypertension appears. At the same time, their salt intake increases dramatically. In the north-eastern district of Japan, where the salt intake averages about 25 grams per day, the prevalence of hypertension is much increased.

In spite of the relationship between blood pressure and sodium intake, no correlation between sodium intake and blood pressure has been demonstrated within Western populations with a sodium intake of 5–15 grams per day.

Recently, Meneely and Batterbee (1976) pointed out that the relationship between salt intake and hypertension depends on the susceptibility of the individual. This susceptibility is explained by genetic factors. According to this theory, only individuals who are genetically equipped to become hypertensive are affected

by an excess salt intake. Moreover, such individuals remain normotensive on a low dietary salt intake. From our material, we have looked for any tendency showing a causal relationship between the salt intake and blood pressure (see Figure 13.3). We could not find any correlation whatever between the salt intake and blood pressure in our material. Neither was there a significant relationship when the quotient between the dietary intake of sodium and potassium was correlated with blood pressure ($r < 0.2$).

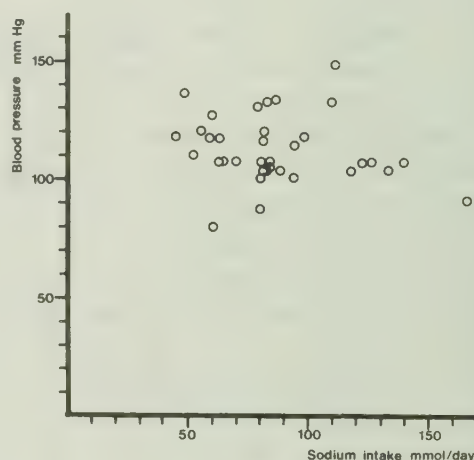


Figure 13.3. The correlation between blood pressure (mm Hg) and sodium intake (mmol/day).

Potassium

CHAPTER 14

POTASSIUM

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An average human body weighing 70 kg contains approximately 3,500 mmol (137 g) of potassium. It is the principal cation of intracellular fluid. Intracellular potassium represents nearly 98 per cent of the body's total potassium (Davidson et al., 1975). The rest of it is extracellular, of which bone contains only negligible amounts. Only 1.4 per cent of the total body potassium is present in the extracellular fluid. The total potassium is somewhat less in women and declines slightly with increasing age. It is generally accepted that the normal dietary intake of about 100 mmol (4 g) of potassium per day is more than enough to meet the daily requirement. Primary dietary deficiency of potassium in man does not seem to occur. On the other hand, secondary potassium deficiency is prevalent in many conditions. This is due to certain diseases or drug therapy. Treatment with diuretics, corticosteroids and insulin may result in a secondary potassium deficiency. Latent potassium deficiency may become manifest during the initial phase of vitamin B₁₂ treatment in subjects with pernicious anaemia. Potassium deficiency is also seen in protein-losing nephropathy and enteropathy, following prolonged vomiting and diarrhoea, and in certain endocrine disorders. Post-operative infusions with an inadequate supply of potassium may also precipitate a deficiency state in many patients who have lived on a low potassium intake. Subclinical deficiency of potassium is, however, difficult to diagnose because most of the potassium is intracellular and

serum levels of potassium are a poor indicator of the total potassium status (Editorial, *British Medical Journal*, 1967). Even other clinical parameters, like ECG changes, correlate poorly with total body potassium. The most reliable indicator of the potassium status is whole-body counting, which is hardly applicable under routine conditions.

Recent studies by Judge (1972), as well as others (Kaul et al., 1965; Dall et al., 1971), indicate that a dietary deficiency of potassium does occur in the elderly in some European communities. Our study regarding the dietary intake of potassium in pensioners who were all living a normal life in their homes was proposed mainly because of the need for information about the intake in a group that could be expected, sooner or later, to need diuretic and other drugs.

MATERIAL AND METHODS

Fat-free and lyophilized food samples, prepared as described in Chapter 3, were used. A total of 115 daily portions from 17 men and 136 daily portions from 20 women were analysed. Wet-ashing was performed in concentrated acids as described in Chapter 13.

The potassium content was determined by flame photometry (Instrument Laboratories Inc., Model 343). By comparing the potassium content in 10 homogenates before and after fat extraction and lyophilization, a 12 per cent de-

crease of potassium was observed in the fat-free lyophilized food homogenates. We have not made any correction for this in the final calculations presented in the tables.

Furthermore, the high temperature during wet-hydrolysis results in additional losses of potassium, which have been estimated to be 5 per cent. Recently, we found that potassium can be extracted more directly by shaking the fat-free, lyophilized food powder with a 2 per cent solution of nitric acid. Although organic bound potassium cannot be extracted with this procedure, we obtained 100 per cent potassium as compared with the wet-ashing procedure, thus indicating losses during the wet-ashing procedure.

RESULTS

Figure 14.1 and 14.2 and Tables 14.I and 14.II show the complete results. The median intake of potassium in the male pensioners was 53 (42–83) mmol, whereas the female pensioners had a median intake of 44 (28–66) mmol. The sex difference with respect to potassium intake was small when expressed in mmol per 1,000

kcal or 10 MJ (see Tables 14.I and 14.II). Only six out of the 17 male and three out of the 20 female pensioners had a potassium intake over 60 mmol. In Figure 14.2 the distribution of potassium in the individual diets is shown for both men and women.

DISCUSSION

An ordinary diet contains 50–100 mmol potassium, which is completely absorbed in the gastro-intestinal tract. Under normal circumstances, nearly 90 per cent of the ingested potassium is excreted in the urine, 5–10 per cent in the faeces and about 5 per cent in the perspiration. When the dietary intake is greatly reduced, potassium deficiency develops only slowly (Judge, 1972). It takes 2–3 weeks for the kidney to adapt to large increases or decreases in the potassium load. The conservation capacity of the kidney for potassium is rather poor even when the dietary intake is low. This is in contrast to the kidneys' ability to produce sodium-free urine when the intake is poor or the elimination is increased via other routes (Judge, 1972; Davidson et al., 1975).

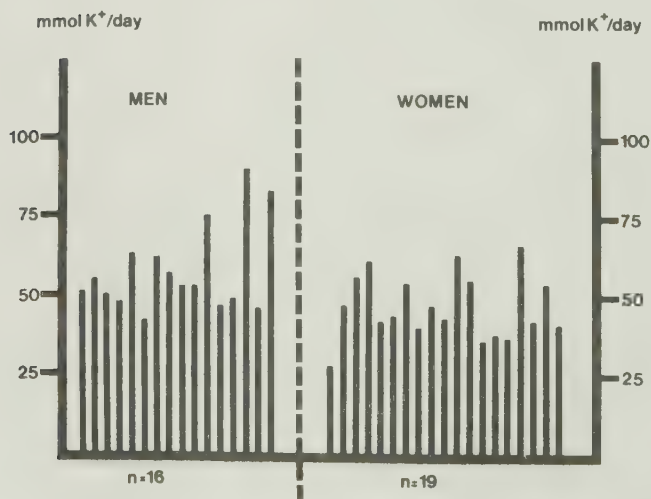


Figure 14.1. The median intake of potassium (expressed in mmol/day) by the male and female pensioners.

Table 14.I. Potassium intake in the male pensioners (mmol/day)

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
002	48	47	49	58	51	62	56	51	47– 62
005	49	62	44	55	63	78	53	55	44– 78
017	50	64	57	48	46	46	53	50	46– 64
026	33	48	39	88	65	59	38	48	33– 88
032	56	73	72	46	47	63	94	63	46– 94
036	46	50	54	27	32	27	42	42	27– 54
037	62	61	71	70	59	77	42	62	42– 77
038	49	57	71	64	52	55	59	57	49– 71
042	55	80	79	50	53	45	34	53	34– 80
044	47	66	49	59	53	53	62	53	47– 66
045	72	81	75	63	83	78	67	75	63– 83
056	36	48	47	48	44	53	42	47	36– 53
060 ¹	—	—	—	84	100	91	—	—	84–100
067	52	42	61	55	49	40	37	49	37– 61
088	69	46	69	106	90	122	99	90	46–122
090	42	51	61	43	46	46	43	46	42– 61
096	83	75	86	81	78	83	112	83	75–112

Mean 62 mmol (30 mmol/1,000 kcal or 72 mmol/10 MJ)
Median 53 mmol (26.5 mmol/1,000 kcal or 63 mmol/10 MJ)
n = 112 daily food portions

S.D. 25 mmol
Range 42–90 mmol

Table 14.II. Potassium intake in the female pensioners (mmol/day)

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
003	26	34	25	28	24	31	39	28	24–39
008	49	46	51	44	48	43	47	47	43–51
010	62	53	56	52	61	52	56	56	52–62
013	55	61	52	69	69	65	44	61	44–69
028	48	42	37	48	35	47	36	42	35–48
034	48	62	40	40	47	42	44	44	40–62
035	57	46	55	54	60	40	52	54	40–60
039	42	37	36	41	35	40	46	40	35–46
049	52	48	55	45	32	43	47	47	32–55
054 ¹	—	—	—	44	44	46	—	—	—
057	41	38	53	59	48	42	43	43	38–59
064	57	67	59	71	67	62	60	62	57–71
065	55	64	67	55	57	54	52	55	52–67
069	44	36	34	43	43	36	35	36	34–44
070	27	31	47	43	25	45	38	38	25–47
076	31	37	34	44	45	49	23	37	23–49
080	54	66	68	75	74	36	49	66	36–75
081	44	42	29	36	42	39	44	42	29–44
082	57	64	65	54	47	47	20	54	20–65
084	38	42	43	41	27	41	37	41	27–43

Mean 47 mmol (28.5 mmol/1,000 kcal or 68 mmol/10 MJ)
Median 44 mmol (27.5 mmol/1,000 kcal or 65 mmol/10 MJ)
n = 153 daily food portions

S.D. 12 mmol
Range 28–66 mmol

¹ Excluded from the calculation of median, mean, standard deviation and range for the whole group.

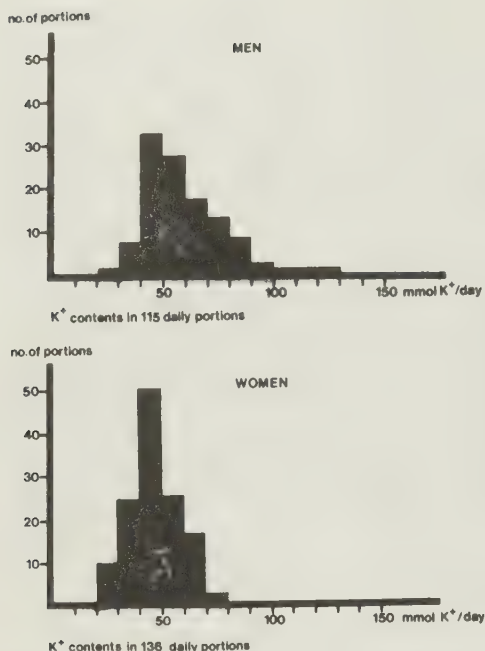


Figure 14.2. The distribution of potassium (mmol/day) in individual diets of the male and female pensioners.

Under normal conditions, a dietary potassium deficiency is extremely rare. Many common food items are rich in potassium. Abramson (1975) gives the potassium content, in mg per 100 g, of the following foodstuffs: potatoes (150 mg), green vegetables (200–600 mg), fish (310–400 mg), meat (300–400 mg) and milk (150 mg). Abramson also reported the following values, in mg, for brown beans (1,300 mg) and yellow peas (910 mg). The last two food items were generally consumed once a week earlier in Sweden, but such a tendency was less frequent among the pensioners, as was evident from their menus.

Judge (1972) pointed out, however, that a dietary deficiency of potassium is frequent in the elderly. His findings were based on several studies in a British population. At present, there are no internationally accepted standards

for the minimum daily requirement of potassium, and it is thus difficult to say at what levels of dietary intake a true potassium deficiency may occur. Balance studies show that a minimum intake of 65 mmol per day is required (Weisberg, 1953). Judge (1972) recommends a minimum daily intake of 65 mmol. If we accept 65 mmol as the normal daily requirement, which is also in accordance with the U.S. recommendations (*Recommended Dietary Allowances*, 1974), the intake of potassium by the present group was low, even after making the necessary corrections for the losses that occurred during the preparation of the daily portions prior to analysis. The female pensioners appeared to have a particularly low intake, also compared with a female group, 55–79 years old, participating in a blood pressure investigation (Thulin 1977), whose urinary potassium excretion was found to be 55 mmol/day on an average, using the double portion technique. (See also Chapter 3, Table 3.IV) In the population study of 70 years old people in Gothenburg (Sweden), B. Steen (1977) reported a mean daily intake of 68 ± 25 mmol potassium by the women based on food table calculations. We have no data on the potassium status of our pensioners except that they, during the 6 years of observations, were living a normal life other than the exceptions mentioned in Chapter 6.

Judge (1972) found in his investigation that the low intake of potassium by the elderly in Great Britain was due to a low consumption of potatoes and milk – two staple food items in most European communities. Judging from the lactose intake (Chapter 11), the milk consumption by the pensioners was on an average 250–350 ml milk daily, which is more than we found in a younger age group (Borgström et al., 1975). In addition, the pensioners consumed reasonable quantities of potatoes and fish as recorded in the menus.

As pointed out earlier, the uncertain basis

for recommending a minimum daily requirement makes it difficult to draw any conclusions from our study as regards the potassium status of the pensioners. It is, however, important to be aware of the low intake when undue sensitivity to certain drugs is observed in elderly persons. This is especially relevant for digitalis. Prolonged diuretic therapy is another well-known cause of potassium depletion. The im-

portance of potassium supplementation in connection with diuretic therapy has recently been discussed (Wilkinson et al., 1975; Abdulla et al., (1975 a). Because cardiovascular disorders are rather frequent in the elderly and because prolonged diuretic therapy is practised very often, it is also important to consider the dietary intake of electrolytes, especially potassium.

Calcium

CHAPTER 15

CALCIUM

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CHAPTER 15

CALCIUM

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Calcium constitutes 1.5 to 2 per cent of the total body weight. An average adult's body contains approximately 1,200 g calcium, and 99 per cent of this is in the skeleton. The small amount of ionized calcium, which is found in body fluids, is essential for blood coagulation, neuromuscular activity and enzymatic and secretory processes (Hegsted, 1973). The serum calcium level is under homeostatic control and is regulated by the parathyroid hormone, calcitonin, metabolites of vitamin D and the dietary calcium.

Calcium absorption is influenced by the presence of many substances in the diet, including other divalent metals. Dietary fat, oxalic acid and phytic acid are known to inhibit the absorption of calcium, whereas lactose and vitamin D have enhancing effects (McBean and Speckman, 1974). The ability to absorb dietary calcium declines markedly with increasing age (Bullamore et al., 1970; Nordin et al 1972). Such a decreased absorption may be related to a mild deficiency of vitamin D or possibly a defective vitamin D metabolism (Nordin, 1974). On the other hand, the dietary calcium level may interfere with the absorption of other divalent metals, like magnesium, zinc and copper (Underwood, 1971).

In recent years a calcium-binding protein has been isolated in the mucosa of the jejunum in several species including man (for references, see Morrissey et al., 1975). This protein participates in calcium absorption. Animal studies have demonstrated that the protein is involved

in the adaptation phenomenon in which the intestine's ability to absorb calcium changes in response to the calcium intake. In the presence of dietary vitamin D, a reduction in calcium intake is associated with an increase in both calcium-binding protein and in the percentage of the calcium that is absorbed. This increase is greater in rapidly growing than in adult animals. This may have some significance on the observed fall in calcium absorption with advanced age in man. In contrast to several animal studies, the vitamin D dependence of the intestinal calcium-binding protein has not been established in man (Morrissey et al., 1975).

Osteoporosis in the elderly is a major problem of calcium metabolism. It has been suggested that osteoporosis is a consequence of either a dietary calcium deficiency or a negative calcium balance resulting from poor absorption (Nordin, 1974). The present study has been conducted in order to determine the dietary intake of calcium by the pensioners and, secondly, to compare this with the dietary intake of calcium by a younger population studied previously.

MATERIAL AND METHODS

Analyses were performed on lyophilized, fat-free, food homogenate prepared as described in Chapter 3. Duplicates of 0.5 g were suspended in glass-bi-distilled water and wet-ashed in concentrated acids — sulphuric, nitric and per-

chloric acids — as described in Chapter 13. The calcium content was determined with flame photometry (Eppendorf). The values were calculated from a standard curve of diluted calcium nitrate (analytical grade, Kebo, Sweden). The standard error of the mean based on 10 determinations was estimated to be ± 0.3 per cent.

As we found in an earlier study regarding the calcium intake in a younger population, no significant difference could be demonstrated by analysing the material with atomic absorption spectrophotometry and flame photometry (Jägerstad et al., 1975). Furthermore, there were no differences in the calcium content between the fresh homogenated material and the corresponding lyophilized powder.

The calculation of the statistical correlations was performed as described by Dixon and Massey (1957).

RESULTS

The daily intake of calcium for the 67–69-year-old male pensioners was 726 mg (18 mmol), expressed as median for the whole group. The individual median intake, based on seven whole-day portions, varied between 450 mg (11.2 mmol) and 1,130 mg (28.2 mmol) (Table 15.I and Figure 15.1). Corresponding values for the women were 610 mg (15.1 mmol), range 370–830 mg (9.2–20.7 mmol), (Table 15.II and Figure 15.1). In Figure 15.1 the median intake of calcium in 10 men and 10 women, between 25 and 60 years of age, from an earlier study is also shown. The younger male adults had a higher calcium intake than the male pensioners, but the female pensioners consumed slightly more than the younger women. The dispersion of the daily intake, as regards calcium, is demonstrated in Figure 15.2.

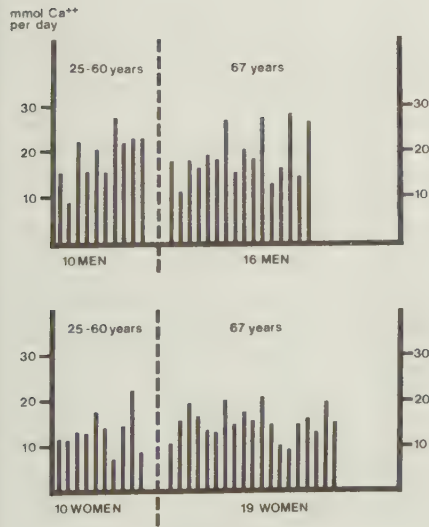


Figure 15.1. The median daily intake of calcium, expressed in mmol, by 16 male and 19 female 67-year-old pensioners and by 10 men and 10 women aged 25–60 years.

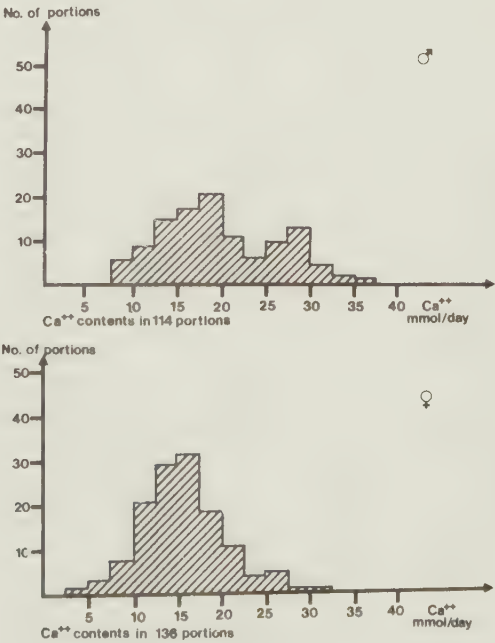


Figure 15.2. The dispersion in the daily intake of calcium by male pensioners (upper figure) and by female pensioners (lower figure).

Table 15.I. The daily intake of calcium, mmol/day, by 67 to 69-year-old male pensioners

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
002	17.2	17.2	18.0	18.0	14.5	23.2	21.0	18.0	14.5–23.2
005	17.5	9.7	7.7	9.0	17.2	25.2	11.2	11.2	7.7–25.2
017	19.5	17.2	17.5	18.2	18.0	11.5	18.0	18.0	11.5–19.5
026	11.5	16.2	11.0	19.0	21.7	20.2	8.5	16.2	8.5–21.7
032	19.2	29.7	24.7	13.2	23.0	16.5	18.2	19.2	13.2–29.7
036	18.2	25.7	22.7	14.2	11.0	13.2	18.5	18.2	11.0–25.7
037	28.9	25.0	34.1	34.1	21.5	30.0	21.5	27.0	21.5–34.1
038	13.7	15.2	25.2	11.5	13.0	17.7	16.7	15.2	11.5–25.2
042	21.7	21.2	29.9	19.0	20.5	19.5	12.5	20.5	12.5–29.9
044	13.0	19.2	19.0	15.5	18.5	20.5	15.2	18.5	13.0–20.5
045	27.7	28.4	27.7	22.7	25.0	25.7	27.4	27.4	22.7–28.4
056	10.0	17.7	21.7	9.5	8.5	12.7	12.7	12.7	8.5–21.7
060 ¹	—	—	—	36.4	32.0	25.7	—	—	25.7–36.4
067	17.2	16.5	28.7	15.0	17.5	14.2	15.5	16.5	14.2–28.7
088	28.2	16.2	26.2	30.0	30.7	34.2	23.7	28.2	16.2–34.2
090	13.5	15.2	20.7	14.5	10.2	15.5	12.5	14.5	10.2–20.7
096	29.7	26.2	29.2	26.4	27.4	27.4	35.9	26.4	26.2–35.9
Mean	16/112	19.6 (784 mg)			Median	18.1 (726 mg)			
S.D.	16/112	6.6 (264 mg)			Range	11.2–27.4 (450–1,100 mg)			

Table 15.II. The daily intake of calcium, mmol/day, by 67 to 69-year-old female pensioners

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
003	9.0	10.5	9.7	9.5	15.0	15.7	15.7	10.5	9.0–15.7
008	18.2	12.0	12.7	16.5	15.5	17.5	12.5	15.5	12.0–18.2
010	19.5	19.5	19.2	19.2	16.2	14.5	21.7	19.5	14.5–21.7
013	22.2	12.0	16.5	16.7	19.7	13.7	16.2	16.5	12.0–22.2
028	14.7	15.0	10.2	12.7	13.2	16.0	10.2	13.2	10.2–16.0
034	15.0	18.5	10.7	12.5	14.7	13.0	9.5	13.0	9.5–18.5
035	27.2	25.0	26.9	18.5	19.0	16.0	20.0	20.0	16.0–27.2
039	14.7	13.0	12.2	13.5	15.2	16.5	17.5	14.7	12.2–17.5
049	25.7	14.0	17.5	19.7	20.2	15.0	12.2	17.5	12.2–25.7
054 ¹	—	—	—	10.5	17.2	14.2	—	14.2	10.5–17.2
057	14.5	14.0	16.7	11.7	17.5	15.5	23.0	15.5	11.7–23.0
064	27.7	20.0	20.7	20.0	23.5	16.5	22.2	20.7	16.5–27.7
065	13.7	18.2	20.0	12.2	14.5	16.0	13.2	14.5	12.2–20.0
069	11.7	8.5	6.7	10.0	13.0	11.2	10.0	10.0	6.7–13.0
070	8.5	9.2	14.5	7.2	4.2	12.5	10.0	9.2	4.2–14.5
076	10.2	12.2	13.7	16.7	16.5	12.2	6.5	12.2	6.5–16.7
080	15.5	23.5	31.9	20.2	16.0	14.2	15.5	16.0	14.2–31.9
081	13.0	14.7	13.0	11.0	14.2	11.0	15.7	13.0	11.0–15.7
082	23.2	19.7	19.7	17.2	25.0	17.2	17.0	19.7	17.0–25.0
084	11.0	12.7	15.7	22.0	15.2	19.2	13.2	15.2	11.0–22.0
Mean	19/133	15.6 (624 mg)			Median	15.2 (610 mg)			
S.D.	19/133	4.7 (188 mg)			Range	9.2–20.7 (370–830 mg)			

¹ Excluded from the calculation of the mean value, median and S.D. for the whole group.

About 10 per cent of the diets of the male subjects and 20 per cent of the diets of the female subjects contained less than 500 mg (12.5 mmol) of calcium. When expressed per 1,000 kcal (4.2 MJ), the average daily intake was 370 mg (9 mmol) for both the men and women.

DISCUSSION

In Sweden, milk and dairy products, and especially cheese, are the predominant sources of calcium, and are estimated to provide 75 per cent of the dietary calcium (G. Krantz, Swedish Dairy Cooperation, 1977). The quantities of the different food items were not known in the present study. From the intake of lactose it was, however, possible to estimate the contribution of calcium from milk consumption (see Chapter 14), which was approximately half of the daily intake. Based on the lactose intake, the male pensioners consumed, on an average, 360 ml of milk daily and the female pensioners 270 ml. In the younger population corresponding figures were 300 and 200 ml, respectively (Borgström et al., 1975).

The daily dietary requirements of calcium have provoked considerable controversy for a number of years. The FAO/WHO Expert Group (1962) on calcium requirements suggested that an intake between 400 mg (10 mmol) and 500 mg (12.5 mmol) per day would be adequate. This was based on the fact that both children and adults in populations with intakes of calcium as low as 300 mg (7.5 mmol) to 400 mg (10 mmol) were apparently free from signs of calcium deficiency. In *Recommended Dietary Allowances* (1974) a daily intake of 800 mg (20 mmol) of calcium is recommended for adult males and females. Both organizations recommend higher figures for pregnant and lactating women. The higher allowances recommended by the American organization were justified on the basis that large

percentages of the population had at that time intakes of calcium comparable to the higher values. The Swedish recommendation is at present set at > 600 mg per 1,000 kcal (*Kost och Motion*, 1975).

Several recently published studies have found that urinary calcium significantly increased when the protein intake was raised (Johnson et al., 1970; Walker et al., 1972; Margen et al., 1974; Chu et al., 1975). Chu et al. (1975), in their study, found that the increase in urinary calcium is not likely to result solely from enhanced intestinal absorption, but suggest that increased glomerular filtration with

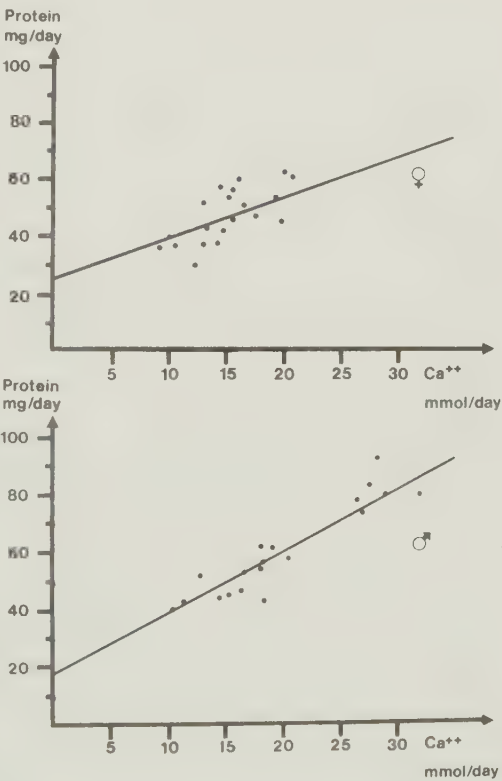


Figure 15.3. Correlation between protein and calcium intake.

Women: $Y = 0.0353 X + 25.58$ $r = 0.59$ $n = 20$
Men: $Y = 0.0538 X + 17.79$ $r = 0.79$ $n = 17$

possible inhibition of renal tubular reabsorption of calcium may be an additional mechanism responsible for the calciuretic effect during high protein intake. The possible significance of high protein and low calcium intake on the development of osteoporosis is stressed.

It is noteworthy that most communities with a low calcium intake also have a low protein intake. The diets in the present study also show a high correlation between calcium and protein intake (Figure 15.3) $r = 0.79$ and $r = 0.59$ for men and women, respectively, which is highly significant ($p < 0.001$). The protein intake (see Chapter 10) varied between 30 and 90 g per day. Walker and Linkswiler (1972) have shown that with protein intakes of 47, 95 and 147 g daily and a constant calcium intake of 800 mg (20 mmol) the urinary losses were 207, 303 and 426 mg calcium per day, respectively. Margen et al. (1974) observed an eightfold increase in urinary excretion of calcium when the

daily intake of protein was raised from 0 to 96 g protein regardless of the calcium intake. The increased urinary loss of calcium often seen in elderly persons may also be a result of physical inactivity (Anonymous, 1972).

From the age of 60–65 years, bone degeneration occurs in both sexes and is associated with a progressive rise in the frequency of femoral neck fracture (Knowelden et al., 1964). The average total bone degeneration between middle life and old age is about 15 per cent of the skeleton, but different bones are affected to different degrees. Most affected is trabecular bone (Trotter et al., 1960). Despite the apparent permanence of the mineral deposits, bone is being constantly formed and resorbed — more rapidly during early development, and at a declining rate during adult life (Picken, 1960). In adults it has been estimated that about 700 mg of calcium enters and leaves the bones each day (Whedon, 1964).

Magnesium

CHAPTER 16

MAGNESIUM

MARGARETHA JÄGERSTAD, MOHAMMED ABDULLA, SVEN SVENSSON
and ÅKE NORDÉN

The adult human body contains 21 to 28 g of magnesium, or about 1,000 mmol (Schroeder et al., 1969).

About 55–60 per cent of the total body magnesium is in the skeleton, where it probably provides the main reserve of the element in the body (*Recommended Dietary Allowances*, 1974). Among other tissues the muscles have the highest magnesium content. The skeletal muscles and the heart muscle contain 10.5 and 6.5–11 mmol/kg, respectively (Szelényi et al., 1973).

Only approximately 1 per cent of the total body content of magnesium is extracellular. The intracellular concentration of magnesium is more than 10 times higher than that outside the cell, and this is maintained even when the extracellular concentration falls very low. Next to potassium, magnesium is the predominant cation in the cells and is an essential part of many enzymes involved especially in carbohydrate metabolism. It is also important for maintaining electrical potential in nerves and muscle membranes.

Intestinal absorption of magnesium appears to be an active process that, in part, is affected by an independent mechanism with regard to calcium uptake. (MacIntyre and Robinson, 1969, *Report of the ARC/MRC Committee*, 1974). The ileum is the main site of absorption. The absorption of magnesium does not seem to require a specific factor comparable to the role played by vitamin D for the absorption

of calcium. Several studies using radioactive magnesium suggest that the absorption of magnesium in man is influenced by the load presented to the intestinal mucosa. Thus, on a low magnesium diet a higher percentage is absorbed than after ingestion of a high magnesium diet, but the mechanism of regulation of the gastrointestinal uptake of magnesium is poorly understood (Aikawa, 1976).

Disturbances in magnesium metabolism are not too uncommon in man as a secondary phenomenon to other diseases. The principle causes are parenteral feeding for prolonged periods, failure of absorptive processes for various reasons, renal wastage from drugs, chemicals or kidney diseases (Flink, 1976).

Magnesium deficiency in man leads to neuromuscular dysfunction as manifested by hyperexcitability with tremor, convulsions and diarrhoea and is sometimes accompanied by behavioural disturbances. In experimental animals, magnesium deficiency also affects protein and carbohydrate metabolism and reduces the rate of tissue-component synthesis. Furthermore, potassium is lost from many soft tissues in magnesium deficiency; and even if additional potassium is provided, it cannot be retained and properly utilized. The metabolic relationship to other elements, such as calcium and potassium, as well as its possible significance in cardiovascular disease, makes it important to know the daily intake of magnesium in elderly subjects.

MATERIAL AND METHODS

Fat-free lyophilized powder, prepared as described in Chapter 3, was used. Duplicates of 0.5 g were wet-ashed in concentrated acids: nitric, sulphuric and perchloric acids as described in Chapter 13.

Magnesium was determined by atomic absorption spectrophotometry (Instrumental Laboratory IL 151/251). As a standard, a solution of MgCl_2 for atomic absorption spectrophotometry was used (BDH Laboratory Chemicals Ltd). A comparison between the magnesium content in homogenized samples before and after fat extraction and lyophilization showed a 10 per cent difference, indicating losses during these procedures.

RESULTS

The median daily intake, based on 7-day collections, by 16 men was 210 mg (8.7 mmol), range 150–290 mg (6.2–11.9 mmol), which is shown in Table 16.I and Figure 16.1. The corresponding median intake by the 19 women is presented in Table 16.II and Figure 16.1 and was 170 mg (7.0 mmol), range 100–250 mg (4.1–

10.3 mmol). About one-third of the diets consumed by the male pensioners and two-thirds of the diets consumed by the women contained less than 8.2 mmol (200 mg) (Figure 16.2). From the same figure, the dispersion in the magnesium content of the diets is demonstrated. The range in the 114 male diets was between 4.1 and 18.5 mmol (100–450 mg). The corresponding range for the women diets was 3.3 to 11.5 mmol (80–280 mg).

DISCUSSION

Magnesium occurs widely in foods, particularly in those of vegetable origin. Highly purified foods originating from sugar, starch and fat contain very little magnesium. Some common foods can be ranked in order of decreasing mean concentrations of magnesium as follows: cacao powder, 170 mmol/kg; nuts, 80; cereals, 33; seafoods, 15; meats, 11; legumes, 10; vegetables, 7; cow's milk, 5; fruits, 3; refined sugar, 2; fats, less than 1 (Aikawa, 1976).

According to Jones (1967) the optimal daily intake of magnesium is 300 mg (12 mmol), or 0.17 mmol/kg per day. The Food and Nutritional Board, in *Recommended Dietary*



Figure 16.1 The median intake of magnesium in mmol/day by 17 male and 20 female pensioners.

Table 16.I. The daily intake of magnesium, in mmol/day, by the male pensioners

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
002	6.6	7.8	9.0	9.9	7.0	9.5	9.9	8.4	6.6– 9.9
005	9.5	9.9	5.8	9.0	9.5	11.1	7.4	9.5	5.8–11.1
017	7.8	9.1	10.3	7.0	7.4	7.8	8.2	7.8	7.0–10.3
026	4.1	6.2	6.2	13.6	7.4	6.6	4.5	6.2	4.1–13.6
032	11.1	14.8	10.7	8.2	9.9	10.7	18.5	10.7	8.2–18.5
036	7.8	10.3	11.9	4.5	5.3	5.3	8.2	7.8	4.5–11.9
037	9.9	11.1	12.3	11.5	8.6	9.9	7.0	9.9	7.0–12.3
038	6.6	7.8	11.5	8.6	8.2	11.1	8.6	8.6	6.6–11.5
042	8.6	10.7	12.3	7.8	7.8	7.0	5.8	8.2	5.8–12.3
044	7.8	11.5	8.6	8.6	8.2	9.5	9.1	8.6	7.8–11.5
045	10.5	13.9	11.4	8.5	13.4	11.1	11.1	11.1	8.5–13.9
056	7.0	7.8	7.8	7.4	5.8	7.8	6.6	7.4	5.8– 7.8
060 ¹	–	–	–	12.8	12.8	10.3	–	12.8	10.3–12.8
067	9.5	11.5	10.7	11.5	9.0	9.0	9.1	9.5	9.0–11.5
088	11.9	6.6	9.5	14.8	14.4	14.8	11.5	11.9	6.6–14.8
090	8.2	8.6	9.0	5.3	10.3	7.4	7.4	8.2	5.3–10.3
096	13.6	13.2	14.4	14.0	12.8	12.8	17.7	13.6	12.8–17.7
Mean 16/112	230.9	(9.5 mg)						Median 8.6	(209 mg)
S.D. 16/112	65.6	(2.7 mg)						Range 6.2–13.6	(151–331 mg)

Table 16.II. The daily intake of magnesium, in mmol/day, by the female pensioners

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
003	3.7	5.3	4.1	3.7	3.7	5.3	5.8	4.1	3.7– 5.8
008	8.6	7.4	7.4	6.6	7.4	6.2	7.0	7.2	6.2– 8.6
010	7.8	7.4	7.4	5.8	7.0	5.3	6.6	7.0	5.3– 7.8
013	9.1	9.9	8.2	10.7	10.3	9.9	7.4	9.9	7.4–10.7
028	6.6	7.0	6.6	6.2	6.2	7.8	5.3	6.6	5.3– 7.8
034	6.6	9.5	6.2	6.6	7.0	6.6	6.6	6.6	6.2– 9.5
035	9.0	7.8	8.2	8.6	9.0	7.1	8.6	8.6	7.1– 9.0
039	7.4	7.0	6.6	6.6	7.0	6.6	7.8	7.0	6.6– 7.8
049	9.0	7.4	9.0	7.4	7.8	8.2	7.8	7.8	7.4– 9.0
054 ¹	–	–	–	7.8	7.0	6.2	–	7.0	6.2– 7.8
057	6.6	6.6	8.6	9.1	9.0	6.6	7.0	7.0	6.6– 9.1
064	11.5	11.1	8.6	9.1	10.3	9.0	9.1	10.3	8.6–11.5
065	7.4	7.4	7.8	7.8	4.9	7.4	7.4	7.4	4.9– 7.8
069	5.3	3.7	4.5	5.3	6.6	5.8	4.5	5.3	3.7– 6.6
070	5.3	6.2	8.6	7.4	4.5	7.0	7.0	7.0	4.5– 8.6
076	6.2	4.9	5.3	8.6	5.8	7.8	3.3	5.8	3.3– 8.6
080	7.4	9.5	9.4	10.7	8.6	7.8	7.0	8.2	7.0–10.7
081	6.4	6.2	4.9	4.9	5.8	5.3	6.2	5.6	4.9– 6.4
082	9.0	9.5	9.0	8.6	8.2	8.6	7.8	8.6	7.8– 9.5
084	5.8	6.2	8.6	5.8	4.5	5.3	6.6	5.8	4.5– 8.6
Mean 19/133	175.0	(7.2 mg)						Median 7.0	(170 mg)
S.D. 19/133	41.3	(1.7 mg)						Range 4.1–10.3	(100–250 mg)

¹Excluded from the calculation of the mean, S.D., median and range for the whole group.

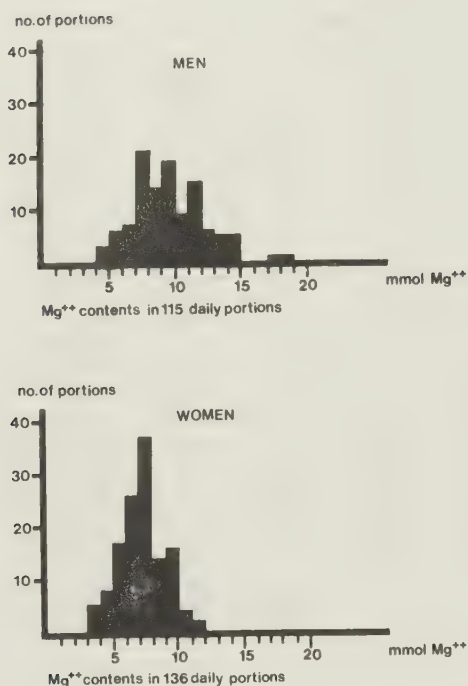


Figure 16.2. The distribution of the magnesium content of the 115 male pensioners' and 136 female pensioners' diets.

Allowances (1974), recommends a daily intake of 350 mg (14 mmol) for adult males and 300 mg (12 mmol) for adult females. The World Health Organization (FAO/WHO, 1973a) recommends a daily intake of 120 mg (5 mmol) magnesium/1,000 kcal (4.2 MJ). In the present study the diets provided, on an average, 105 mg (4.2 mmol)/1,000 kcal for both the male and female pensioners without corrections for losses during analysis. Nicolaysen (1969) estimated that the average Norwegian diet provides 130 mg magnesium per 1,000 kcal. When reviewing the literature regarding nutritionally induced magnesium deficiency in man, Nicolaysen (1969) concluded that a daily intake between 120 and 140 mg (5–6 mmol), or even less, may meet the daily requirements.

When the dietary intake of magnesium is

reduced, the urinary excretion of magnesium is rapidly lowered. The renal mechanism is efficient enough to conserve all but about 0.5 mmol of magnesium per day. At the same time, the gastro-intestinal absorption increases in efficiency (Barnes et al., 1958). Other nutrients that may affect the daily requirements of magnesium are a large intake of protein and calcium, both of which interfere with the absorption of magnesium. In experimental animals, magnesium depletion is enhanced by high protein and calcium intake (Bunce et al., 1963; Colby and Frye, 1951). A high calcium intake may also retard the intestinal absorption of other elements (Skoryna et al., 1971; see Varo, 1974).

Varo (1974) found a high, significant correlation between the death rate from ischaemic heart disease and the estimated calcium to magnesium ratios of the average diets in different OECD countries. The Swedish ratio of 3.41 was calculated from national trade and production statistics. In the present study the ratio was found to vary considerably between different individuals from 1.60 to 4.5 in the male subjects, with a median of 3.45. The corresponding figure for the women was 3.58, range 2.8–4.5 (Table 16.III). One male subject, who later died of myocardial infarction, had the lowest calcium to magnesium ratio, 1.60. The other nine subjects who were found to be suffering from cardiovascular disease (Chapter 5) all had ratios above 3.5, with the exception of subject 67, who had a ratio of 2.9. He had aortic stenosis with atrial fibrillation and died of disseminated emboli. Seelig and Heggveit (1974) reviewed the magnesium interrelationship in ischaemic heart disease. He found reason to conclude that magnesium might play an important role by counteracting the adverse effects of excessive intracellular calcium by affecting the retention of intracellular potassium and by maintaining the integrity of subcellular

Table 16.III. The dietary calcium to magnesium ratio (W/W)

Female pensioners		Male pensioners	
Subject no.	Ratio	Subject no.	Ratio
003	4.2	002	3.5
008	3.5	005	2.0
010	4.5	017	3.8
013	2.8	026	4.3
028	3.3	032	3.0
034	4.0	036	3.8
035	3.8	037	4.5
039	3.5	038	2.9
049	3.7	042	4.1
054	3.4	044	3.5
057	3.6	045	4.1
064	3.3	056	2.8
065	3.2	060	1.6
069	3.1	067	2.9
070	2.2	088	3.9
076	3.5	090	2.9
080	3.2	096	3.2
081	3.9		
082	3.8		
084	4.4		
Median	3.58	Median	3.45
Range	2.8–4.5	Range	1.6–4.5

structures.

The present study shows a magnesium intake that is below the recommendations given by experts within the WHO and the Food and Nutritional Board (*Recommended Dietary Allowances*, 1974). When the intake of magnesium is calculated per 1,000 kcal, it is about 10 per cent lower than that recommended by FAO/WHO (1973a). The analysis of magnesium in the present study has been performed in prepared food, which may account for some loss during cooking. In addition, losses during analytical procedures were found to be 10 per cent, which is not included in the final calcu-

lation. The clinical investigation of pensioners has not revealed any signs of overt magnesium deficiency. Because of magnesium storage in the skeleton and the homeostatic regulation of the plasma magnesium level, a deficiency of this element may be difficult to detect in its early stages. Plasma magnesium is a poor indicator of magnesium status. The erythrocyte magnesium level is a much better indicator, because its concentrations are about 10 times that of the plasma. In clinical practice, however, a whole-blood magnesium determination is not made routinely. The clinical signs of magnesium deficiency are not obvious until the deficiency is severe. Prolonged diarrhoea, however, is an important clinical sign that may be a consequence of, as well as a cause of, magnesium deficiency.

When considering the daily requirements of magnesium in the elderly, it must be borne in mind they are often being maintained on a drug therapy that may interfere with magnesium metabolism. Both mercurial and thiazide diuretics increase the urinary excretion of magnesium, as well as that of potassium and sodium. The daily requirement of magnesium may also depend on other nutrients in the food. In the present study the intake of protein and calcium was relatively high, which possibly affected the absorption and utilization of magnesium. In addition, the intake of potassium, which is important for maintaining adequate intracellular levels of magnesium, was low in our subjects.

In sum, as long as there are uncertainties regarding the minimum dietary allowance of magnesium, it is, indeed, difficult to draw any conclusions regarding the magnesium status in elderly persons. It is, however, very important to be on the alert for subclinical deficiencies in old people, especially during various drug therapies.

Zinc

CHAPTER 18

ZINC

MOHAMMED ABDULLA, SVEN SVENSSON and ÅKE NORDÉN

CHAPTER 18

ZINC

MOHAMMED ABDULLA, SVEN SVENSSON and ÅKE NORDÉN

Traditionally, zinc is classified as a trace element in plant and animal nutrition. As early as in 1869, the nutritional importance of zinc for the growth of *Aspergillus niger* was demonstrated (Raulin, 1869), and the essential role of zinc in higher forms of plant life was later confirmed in 1926 (Sommer and Lipman, 1926). The evidence that spoke for the functional role of zinc in higher animals came later from the work of Birckner and others (Birckner, 1919; Todd, Elvehjem and Hart, 1934). The essential role of zinc in human nutrition was described by Pories and Strain (1966) and Prasad (1966). Zinc is present in at least 20 enzymes of vital importance and is involved in a wide range of cellular activities, including nucleic acid and protein synthesis (Kirchgessner, 1977). In vitamin A metabolism, zinc has been found to be essential for the mobilization of vitamin A from the liver and possibly for the synthesis of retinol-binding protein (Smith, McDaniel, Fan and Halstead, 1973; Abdulla, 1974).

Experimental zinc deficiency in animals has resulted in congenital malformations, growth retardation, abnormal bone metabolism, atrophy of the testes and deformative changes in the skin. In man, zinc deficiency has been demonstrated in many diseases. In 1961, Prasad et al. described cases of dwarfism in Iranian boys who had iron-deficiency anaemia and delayed puberty (Prasad et al., 1961). These boys also had the habit of eating clay. Although the anaemia responded to iron therapy, growth retardation and delayed puberty were only improved after zinc therapy. Similar cases also

were reported from Egypt (Prasad et al., 1963). Zinc-deficiency hypogonadism, associated with dwarfism, hepatosplenomegaly and geophagia, is a well-established syndrome today. In patients with leg ulcers, impaired healing and zinc deficiency are correlated (Hallböök and Lanner, 1972; Hæger et al, 1972). Zinc also plays a key role in the treatment of acrodermatitis enteropatica (Monyahan, 1974; Michaelsson, 1974). Recently, it has been shown that zinc has some protective effect against the toxic action of heavy metals, like lead, cadmium and copper (Abdulla and Hæger-Aronsen, 1974; Hæger-Aronsen et al, 1976; Webb, 1972; Abdulla et al., 1976). Experimental studies in animals indicate that zinc plays a very active role in chemical carcinogenesis (Mathur et al., 1978). With the above background about the essential nature of zinc in human nutrition and disease, we undertook to measure the contents of zinc in food consumed by the pensioners in Dalby.

MATERIAL AND METHODS

The collection and preparation of the samples were made as described in Chapter 3. Using sulphuric, nitric and perchloric acids as described in Chapter 13, 0.5 g of the lyophilized powder was wet-ashed. The influence of fat extraction procedure on the total contents of zinc in the daily portions was studied by analysing 55 dietary portions prior to and after fat extraction. Zinc was determined by atomic ab-

sorption spectrophotometry using a Perkin-Elmer 403 instrument after dilution with de-ionized glass-bi-distilled water. A standard solution of zinc acetate for atomic absorption spectrophotometry was used for making the standard curve (BDH Chemicals Ltd, England). A total of 245 daily portions collected by 17 men and 20 women were analysed.

RESULTS

Complete results are presented in Tables 18.I and 18.II and Figures 18.1 and 18.2. The male pensioners had a median daily intake of 124 μmol (8.2 mg), varying between 56 μmol and 311 μmol (3.7–20.4 mg). The female pensioners had a daily median intake of 110 μmol (7.2 mg), with a range of 57–257 μmol . The mean daily intake for the whole group was 125 μmol (8.2 mg) $\pm$ 44 μmol (2.9 mg). The difference between duplicate determinations was within $\pm$ 2.5 per cent. The fat extraction procedure had no significant effect on the total zinc content (the difference was less than 2.5 per cent). Expressed in 1,000 kcal, the mean in-

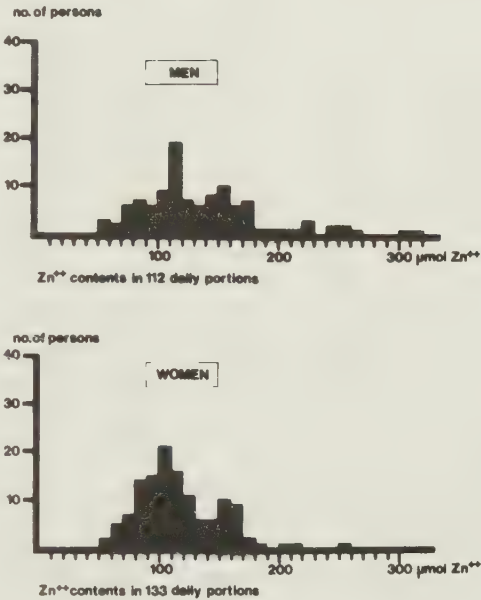


Figure 18.2. The distribution of zinc ($\mu\text{mol/day}$) in individual diets of the male and female pensioners.

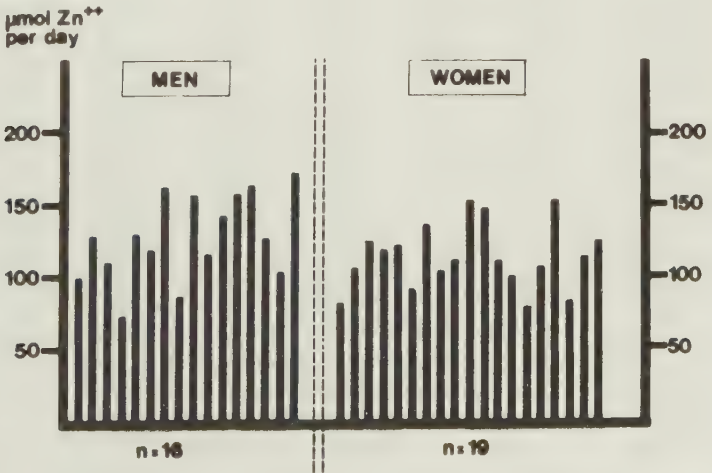


Figure 18.1. The median intake of zinc ($\mu\text{mol/day}$) by the male and female pensioners.

Table 18.I. The daily intake of zinc ($\mu\text{mol/day}$) during a 1-week period in the 67-year-old male pensioners

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
002	119	97	99	116	78	142	90	99	78–142
005	111	107	214	134	123	252	126	126	107–252
017	108	88	117	102	82	149	110	108	82–149
026	58	97	59	125	79	71	56	71	56–125
032	110	157	136	110	128	105	174	128	105–174
036	117	140	148	84	93	87	159	117	84–159
037	263	107	185	152	160	221	140	160	107–263
038	67	79	130	85	87	114	61	85	61–130
042	156	143	197	172	113	123	171	156	113–197
044	100	97	258	113	114	114	175	114	97–258
045	111	168	140	105	140	119	151	140	105–168
056	122	169	157	244	157	200	139	157	122–244
067	152	302	311	172	151	162	140	162	149–311
088	136	79	136	107	125	151	117	125	79–151
090	88	102	100	110	113	78	116	102	78–116
096	229	172	172	169	168	226	241	172	168–241

Mean 16/112 136 μmol (8.9 mg)/day
S.D. 50 μmol (3.3 mg)
Median 124 μmol (8.15 mg)
Range 56–311 μmol (3.7–20.4 mg)

Table 18.II. The daily intake of zinc ($\mu\text{mol/day}$) during a 1-week period in the 67-year-old female pensioners

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
003	82	81	71	58	93	71	111	81	58–111
008	139	130	114	99	105	82	100	105	82–139
010	166	99	157	88	94	123	142	123	88–166
013	162	117	105	256	116	154	97	117	97–256
028	125	105	70	120	169	123	116	120	70–169
034	90	107	108	84	114	81	88	90	81–114
035	152	148	122	134	113	134	220	134	113–220
039	105	100	122	96	102	96	126	102	96–126
049	114	114	105	116	110	94	102	110	94–116
057	151	113	157	110	159	146	151	151	110–159
064	146	140	125	174	169	131	166	146	131–174
065	123	68	108	156	189	88	110	110	68–189
069	105	56	81	99	91	104	81	99	56–105
070	64	70	156	156	62	104	78	78	62–156
076	108	70	67	179	211	105	82	105	67–211
080	152	152	171	142	165	134	120	152	120–171
081	73	82	81	64	85	108	102	82	64–108
082	113	117	104	94	123	134	91	113	91–134
084	165	123	165	105	94	91	160	124	91–165

Mean 19/133 118 μmol (7.7 mg)/day
S.D. 35 μmol (2.3 mg)
Median 110 μmol (7.2 mg)
Range 57–257 μmol (3.7–16.8 mg)

take was 66 and 74 μmol for the male and female pensioners, respectively. We have attempted to determine if there is any correlation between zinc and energy content. We found a weak positive correlation between the two. On the other hand, many low energy portions contained more zinc per unit of energy, and we concluded that the total energy content does not always reflect the intake of the trace elements.

DISCUSSION

Zinc in the normally consumed food of an adult has been estimated to be about 150–230 μmol (10–15 mg) per day, and the effective absorption is believed to be about 10 per cent of this amount (Underwood, 1971). The total amount of zinc found in a 70-kg adult is about 2–2.5 g, which is half that of iron and 10–15 times that of copper. According to older studies, a daily intake of 153 μmol (10 mg) was recommended, whereas recent studies have shown a somewhat higher requirement of 229–306 μmol (15–20 mg) (Mertz, 1974). According to these recommendations, the figures obtained from our investigation are somewhat low. According to two earlier studies, most mixed diets supply 150–230 μmol (10–15 mg) per day (Sanstead et al., 1967; Schroeder et al., 1967). In another study, adult diets were found to provide 76–340 μmol zinc per day (McCance and Widdowson, 1942). This range is in close agreement with our results, although the relative availability of zinc from various foods is not known. Phytate content, calcium, processing, chelates used in canning and certain drugs, such as D-penicillamine and the corticosteroids, are some of the factors influencing the availability of zinc for effective absorption. Modern methods for food processing are accompanied

by a reduction of trace elements compared with the natural material. Whereas raw wheat contains 450 $\mu\text{mol/kg}$ (30 mg/kg), wheat flour contains only 150 $\mu\text{mol/kg}$ (10 mg/kg) of the metal. Galvanized pipes, used in the past for the storage and transport of water, were an additional source of zinc in drinking water. Today, galvanized pipes are seldom used and water drinking has also been drastically reduced. Substitutes as beer and soft drinks are poor sources of zinc. The modern trend to reduce energy intake may also lead to a low zinc intake. Oberleas and Prasad (1969) recommend zinc supplementation in certain foods, which may improve the low dietary intake of zinc in industrialized countries.

Trace element nutrition may be a major problem of old age. It has been estimated that by 1980 20 per cent of the population of most industrialized countries will be composed of subjects over 65 years of age (Carlson, 1972). With advancing age, there is a reduction in energy metabolism. Digestive inefficiency may occur in the elderly. And the human body also loses protein as age advances. Changes in the dental status with increasing age also contribute to changes in dietary habits that may restrict the selection of food. The uncertainty of the recommended values to meet the minimum daily requirement makes it difficult to draw any conclusions regarding the zinc status of the pensioners in the present study. But the follow-up studies have shown that most of the pensioners are in good health; moreover, clinical parameters have not indicated any signs of deficiency for any of the nutrients. A subclinical deficiency, however, of one or another nutrient or of one or more trace elements in the elderly may be far more common in affluent countries than is commonly believed. The implication of this may be of importance when elderly patients are treated with drugs.

Copper

CHAPTER 19

COPPER

MOHAMMED ABDULLA and SVEN SVENSSON

COPPER

MOHAMMED ABDULLA and SVEN SVENSSON

The average adult human body contains 1.3-1.9 mmol (80-120 mg) of copper (Underwood, 1971). In comparison with the amounts of zinc and iron found in the normal body, the copper content is about 20-50 times lower. The major role of copper is related to the activity of oxidation-reduction enzymes of the tissues and in haemoglobin synthesis. Copper is essential for the activities of several enzymes, such as monoamine oxidase, superoxide dismutase, tyrosinase, cytochrome oxidase, uricase and ascorbic acid oxidase. The mechanism of the enzyme action is not well established. In infants, a hypochromic microcytic anaemia is related to a copper deficiency, and is due to low levels of copper in milk. Wilson's disease in man is a well-known condition associated with abnormal copper metabolism. In Menke's kinky hair syndrome, the brain tissues of infants were found to be devoid of cytochrome oxidase activity; also afflicted infants have low serum levels of copper and low ceruloplasmin levels. In animals, anaemia, depigmentation disorders, neonatal ataxia and cardiovascular defects are some of the conditions associated with a dietary deficiency of copper (Underwood, 1971; Prasad, 1976). Although no single criterium is known to be associated with copper deficiency, analysis of cytochrome oxidase, monoamine oxidase and ceruloplasmin have been proposed for the diagnosis of copper deficiency (Kirchgessner et al., 1977). From a nutritional point of view, the deficiency or excess of other metals, such as calcium, magnesium, iron and zinc, can influence the absorption of copper. In the present

study, the daily dietary intake of copper in the pensioners has been measured.

MATERIAL AND METHODS

The collection and preparation of the samples were made as described in Chapter 3. One-half gram of the lyophilized powder was wet-ashed with concentrated sulphuric, nitric and perchloric acids as described in Chapter 13. The copper content was determined by atomic absorption spectrophotometry using a Perkin-Elmer 403 instrument. To construct a standard curve, a solution of copper chloride for atomic absorption spectrophotometry was used (BDH Chemicals Ltd, England). The recovery of the method was studied by adding known amounts of copper to the food portions before wet-ashing.

The effects of fat extraction, on the copper content were studied by wet-ashing 55 dietary portions before and after the fat extraction. A total of 244 fat-free and lyophilized food portions were analysed for copper content.

RESULTS

The difference in duplicate determinations was less than 1 per cent. The average recovery rate of added copper to the food samples was 95 per cent. The losses owing to fat extraction were less than 4 per cent.

The complete results as regards the copper

content in 244 portions of food samples analysed are shown in Tables 19.I, 19.II, and in Figures 19.1, 19.2. The median daily intake of copper in the male pensioners was 19.6 μmol (1.25 mg), varying between 8.9 and 53.1 μmol (0.5-3.3 mg), whereas female pensioners had a median daily intake of 15.2 μmol (0.9 mg), with a range of 7.3-36.4 μmol (0.4-2.3 mg). The mean intake in the total material was $17.9 \pm 6.2 \mu\text{mol}$ ($1.1 \pm 0.4 \text{ mg}$), or 10 μmol (0.6 mg)/1,000 kcal. Expressed in 10 MJ, both men and women consumed 24 μmol (1.5 mg).

DISCUSSION

Balance studies on copper requirement have indicated a daily intake of 31-39 μmol (2-2.5 mg) copper for adults and 0.8 μmol (0.05 mg)/kg body weight for infants and children (Cartwright, 1950; Engel et al., 1967; *Recommended Dietary Allowances*, 1974). Most European diets are assumed to contain sufficient copper to meet the minimum daily requirement. Residents of communities with staple food items, like rice and wheat in their diets, may ingest 63-94 μmol (4-6 mg) of copper per day. Shellfish, oysters, legumes, meat products and dried vegetables are some of the food items that

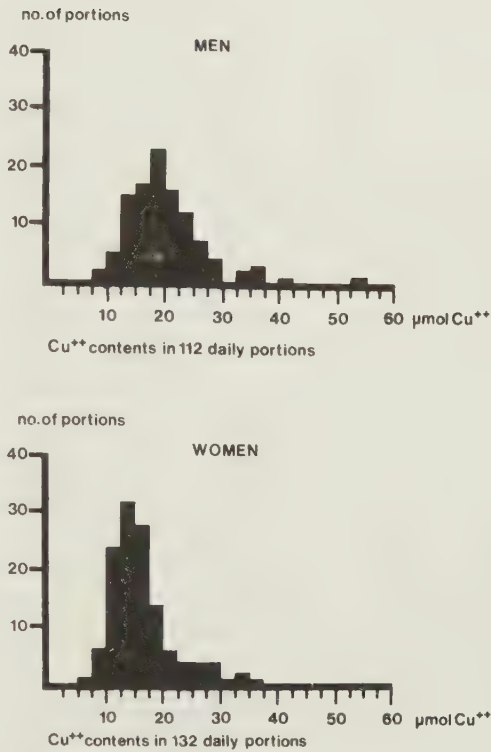


Figure 19.2. The distribution of copper ($\mu\text{mol/day}$) in individual diets of the male and female pensioners.

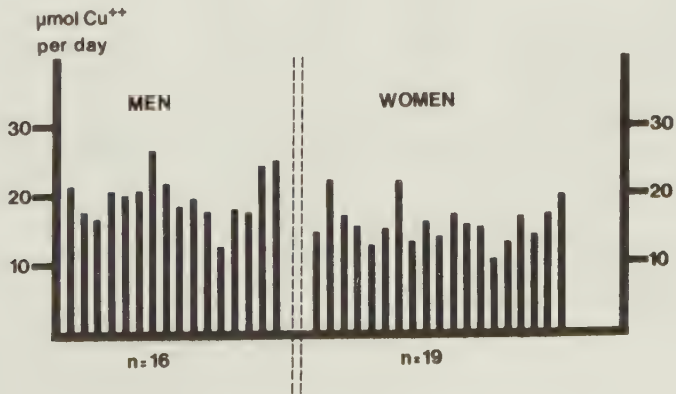


Figure 19.1 The median intake of copper ($\mu\text{mol/day}$) by the male and female pensioners.

Table 19.I. The daily intake of copper ($\mu\text{mol}/\text{day}$) in 67-year old male pensioners

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
002	20.6	21.3	37.1	18.5	19.0	19.3	22.6	20.6	18.5–37.1
005	14.3	27.9	22.3	17.3	16.2	19.0	12.4	17.3	12.4–27.9
017	17.1	16.9	16.5	22.8	12.1	15.2	12.7	16.5	12.1–22.8
026	20.1	17.2	19.8	23.1	16.9	20.7	14.9	20.4	14.9–23.1
032	19.9	18.4	20.2	20.1	16.3	16.6	17.9	19.8	16.3–20.2
036	20.7	18.8	22.4	20.4	14.3	19.8	6.2	20.4	14.3–22.4
037	26.2	29.8	22.8	22.1	25.9	32.8	53.1	26.2	22.1–53.1
038	21.5	24.8	27.6	15.7	18.2	13.8	37.1	21.5	13.8–37.1
042	12.8	15.4	18.8	18.0	20.2	12.5	19.0	18.0	12.5–20.2
044	18.8	18.2	28.1	19.5	15.0	18.2	18.8	19.5	15.0–28.1
045	16.3	19.3	22.9	14.6	16.2	21.5	17.3	17.3	14.6–22.9
056	12.4	16.2	16.2	8.9	10.3	9.5	14.9	12.4	8.9–16.2
067	13.2	23.4	17.9	25.7	22.9	14.7	14.6	17.9	13.2–25.7
088	26.5	17.9	12.1	14.4	16.9	12.5	14.1	14.4	12.1–26.5
090	21.7	24.0	36.4	23.4	20.7	25.6	25.3	24.0	20.7–36.4
096	24.8	15.4	33.6	24.0	22.4	41.0	26.5	24.8	15.4–41.0

Mean: 16/112 20.1 μmol (1.28 mg)/day
S.D. 16/112 6.6 μmol (0.42 mg)
Median 19.0 μmol (1.21 mg)
Range 8.9–53.1 μmol (0.57–3.38 mg)

Table 19.II. The daily intake of copper ($\mu\text{mol}/\text{day}$) in 67-year old female pensioners

Subject no.	M	T	W	Th	F	Sa	Su	Median	Range
003	15.4	19.8	23.9	11.6	13.3	12.7	14.1	14.1	11.6–23.9
008	27.2	15.4	21.7	24.0	28.3	20.7	20.1	21.7	15.4–28.3
010	20.1	31.6	16.6	13.9	13.0	11.4	17.3	16.6	11.4–31.6
013	13.8	25.6	16.5	17.4	8.3	10.6	—	15.2	8.3–25.6
028	12.4	17.7	17.3	8.9	9.5	11.3	15.8	12.4	8.9–17.7
034	15.0	7.7	10.8	16.9	15.4	14.7	13.5	14.7	7.7–16.9
035	29.5	36.4	27.6	21.7	17.1	19.5	16.9	21.7	16.9–36.4
039	14.1	12.4	12.8	12.5	16.2	11.6	12.7	12.8	11.6–16.2
049	25.1	10.0	19.1	17.2	15.7	10.8	13.9	15.7	10.0–25.1
057	19.9	13.3	11.3	14.6	11.6	14.7	9.5	13.3	9.5–19.9
064	16.8	15.0	13.5	20.2	12.1	19.3	16.8	16.8	12.1–20.2
065	14.7	14.6	16.0	11.4	15.4	22.9	19.1	15.4	11.4–22.9
069	12.8	19.1	36.0	14.7	13.5	12.5	15.7	14.7	12.5–36.0
070	11.7	7.8	10.2	13.6	11.1	12.1	7.3	11.1	7.3–13.6
076	15.4	11.7	12.5	11.4	12.8	13.0	18.5	12.8	11.4–18.5
080	17.9	13.5	18.0	17.4	11.1	16.5	13.8	16.5	11.1–18.0
081	12.1	16.5	11.9	11.4	13.8	32.7	13.9	13.8	11.4–32.7
082	19.5	12.8	14.7	17.1	27.6	13.3	16.9	16.9	12.8–27.6
084	22.8	12.2	14.1	15.4	25.4	19.6	19.6	19.6	14.1–25.4

Mean 19/132 16.1 μmol (1.03 mg)/day
S.D. 19/132 5.6 μmol (0.36 mg)
Median 14.9 μmol (0.95 mg)
Range 7.3–36.4 μmol (0.47–2.32 mg)

contain high amounts of copper. Dairy products are rather poor sources of copper. Drinking water is another source of copper in certain areas (see Chapter 25); here the copper in the water results from using copper vessels for storage, as well as from the presence of copper plumbing in the pipes. An investigation conducted in North America regarding the dietary intake of copper in young adults showed an average intake of 60 μmol (3.8 mg) per day (Zook and Lehmann, 1965). The copper content in the diet of different age groups varies little, and copper balance is maintained in adults on an intake of about 31 μmol (2 mg) per day (Leverton and Binkley, 1944; Underwood, 1971).

Like the dietary intake of some of the other elements, such as potassium, magnesium and zinc studied in the present investigation, also the copper content in the dietary portions is low by comparison with the values given by the U.S. Nutritional Board (*Recommended Dietary Allowances*, 1974). We cannot specifically point out any particular cause for the lower intake by the pensioner group. But it is known that the dietary intake and tissue stores of copper decreases with age (Prasad, 1976). In general, the Dalby pensioners consumed considerable quantities of dairy products and refined cereals, which are rather poor sources of copper. No significant losses of trace elements took place during the preparation of samples prior to analysis.

The higher intake of calcium in comparison with the other metals, owing to milk consumption, may aggravate the low copper intake. Sulphur and molybdenum are two other dietary ingredients that influence the absorption of copper.

In most mammalian species, a copper deficiency is mainly manifested by anæmia of varying type and degree. The role of ceruloplasmin in hæmatopoiesis, through the conversion of ferrous iron to the ferric state, is rather well known. The iron status and hæmoglobin levels in the pensioners are reported in Chapter 17. There were no indications of an iron-deficiency anæmia in this group. In our clinical data, ceruloplasmin is not included.

In clinical material, the relationship between copper and zinc in plasma seems to be of great value in judging the extent of deficiency or excess of either of the metals. In practically all diseases where a secondary zinc deficiency is expected, there is a reciprocal increase of copper in the plasma. This is especially seen in malignant diseases. In one clinical study, the oral intake of zinc (70 μmol , or 4.5 mg/day) for 6 weeks resulted in a drastic reduction of copper in the plasma (Abdulla et al., 1976). Copper bound to protein appears to be greatly affected by high tissue concentrations of zinc. Our observations indicate that the copper to zinc ratio in plasma is a much better parameter in the diagnosis of a subclinical deficiency or excess of either of the metals (Abdulla and Ihse, 1976).

Like all other trace metals, the uncertainty of the recommended dietary allowances makes it difficult to draw any conclusions. Since the clinical parameters and follow-up studies have indicated normal functions in most of the subjects investigated, we can conclude that the intake of most trace elements is adequate to meet the minimum daily requirement. It is, however, important to watch for subclinical deficiencies of trace metals in the elderly, which can be aggravated by diseases and drug therapy.

Chromium and Nickel

CHAPTER 20

CHROMIUM AND NICKEL

MOHAMMED ABDULLA and SVEN SVENSSON

CHROMIUM AND NICKEL

MOHAMMED ABDULLA and SVEN SVENSSON

The rapid improvements in analytical methodology during the last few decades have helped to recognize the existence of many hitherto unknown trace elements in living systems. In the field of nutrition, the term "essential" is traditionally assigned to a group of certain organic substances and inorganic elements that fulfill many specialized functions in the organism and have to be provided from dietary sources. In spite of the recent advances in trace-element nutrition research, the proposed biological roles of many trace elements are still hypothetical. The absence of acute and early recognizable symptoms owing to marginal deficiencies of many trace elements makes it even more difficult to recognize their importance. To study new trace-element requirements, highly sophisticated techniques and materials are requisite. Despite these problems, a tremendous breakthrough has been made in the field of trace-element nutrition during the last 20 years. Before 1960, only nine trace elements were known to be essential in animal and human nutrition. Today, the number has risen to 20 and the trend is that it will increase further in the years to come. Many of the trace elements that are essential for important physiological functions are required only in small concentrations, and it is commonly believed that a trace-element deficiency hardly ever occurs in man. The increased consumption of highly refined foods and food analogues in the industrialized countries, however, may well result in marginal deficiencies of certain trace elements (TEMA 3 Symposium, 1978). In this chapter, the im-

portance of chromium and nickel in human nutrition will be discussed along with the dietary intake of these two elements in the diet of the old-age pensioners residing in Dalby, Sweden.

CHROMIUM

Although the biological importance of chromium in plants and micro-organisms was recognized during the early part of 1900s, the primary interest of chromium in higher animals was confined to its toxic properties until recently. It is generally accepted that hexavalent chromium is much more toxic than trivalent chromium, and it is known that prolonged exposure to chromium dust results in an increased incidence of lung cancer (Brinton et al., 1952). It is also well known that occupational exposure to materials containing chromium induces contact allergy in sensitized individuals.

The discovery by Schwartz and Mertz (1959) that trivalent chromium increases the glucose tolerance of rats gave a new dimension to the nutritional importance of chromium. During the last 15 years, several studies have been made to correlate diabetes mellitus with the defective metabolism of chromium. It is well established today that the biologically active chromium is an integral part of "glucose tolerance factor" (GTF) (Mertz et al., 1978). This factor has been identified as the di-nicotinato, tri amino acid, chromium III complex. It has recently been shown that it potentiates the action of insulin in man and animals (Mertz

et al., 1978). It is also known that the oral administration of GTF to chromium-deficient animals improves glucose tolerance (Mertz et al., 1978; Doisy et al., 1976). Recent studies in Turkey have indicated that the administration of chromium compounds to children resulted in beneficial effects on glucose tolerance and growth (Gürson and Saner, 1973). Further, chromium-rich yeast preparations administered to healthy adults have been shown to result in a reduction of serum cholesterol (Doisy et al., 1976; Liu et al., 1977).

MATERIAL AND METHODS

The collection and preparation procedure of the dietary portions are described in Chapter 3. Five to seven daily dietary portions, representing different days of the week, were pooled to represent the average daily dietary intake. A total of 230 samples from 34 pensioners were included in the study. A known amount of the pooled portion (usually 0.5 g) was mixed with 2.5 ml of concentrated nitric acid (analytical grade) in a specially washed test-tube with a slipped stopper, and the mixture was shaken thoroughly to suspend the food material in the acid. This mixture was allowed to hydrolyse slowly for 12–15 hours in an oven at 60°C. Hydrolysis is normally completed after a few hours. The decomposition of nitric acid during the hydrolysis resulted in a loss of about 0.3–0.4 ml. A reaction blank was made by hydrolysing 2.5 ml of concentrated nitric acid without any food sample under the same conditions. After the completion of the hydrolysis, chromium was analysed by atomic absorption spectrophotometry. The necessary dilutions were made prior to the analysis with ion-free water. A recovery study was made by adding known amounts of chromium to a pooled food sample and hydrolysing it under the same conditions as that for the dietary

samples. A standard curve was made from a solution containing known amounts of chromium chloride (analytical grade, BDH Chemicals).

RESULTS

The complete results are shown in Table 20.I and Figure 20.1. The average intake by the male pensioners was 4.4 µmol/day (229 µg), with a range of 2.5–11.3 µmol (130–588 µg). Corresponding figure for the women was 2.8 µmol/day (145 µg) and 0.84–4.9 µmol (44–

Table 20.I. The daily intake of chromium (µmol/day)

Men no.	Chromium	Women no.	Chromium
002	2.75	003	0.84
005	4.48	008	4.54
017	4.50	010	3.46
026	3.90	013	3.79
032	3.15	028	1.33
036	2.52	034	2.19
037	3.89	035	4.54
038	2.04	039	3.89
042	11.27	049	2.35
044	4.07	057	4.10
056	5.04	064	2.13
067	3.73	065	1.63
088	9.07	069	1.31
090	3.04	070	1.23
096	2.76	076	2.77
		080	4.85
		081	3.37
		082	2.98
		084	2.38
n = 15		n = 19	
Mean 4.4	(229 µg/day)	2.8	(145 µg/day)
Range 2.5–11.3		0.84–4.9	
	(130–588 µg/day)	(44–252 µg/day)	
Mean/1,000 kcal			
2.23	(116 µg/day)	1.77	(92 µg/day)
		n = 34	
		Mean 3.5	(182 µg/day)
		Range 0.8–11.3	(44–588 µg/day)

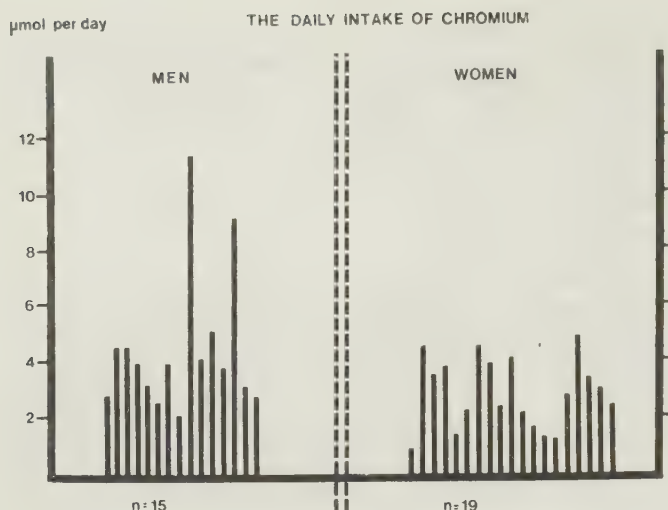


Figure 20.1. The daily intake of chromium by 34 Dalby pensioners.

252 μg), respectively. The recovery study indicated that the losses during hydrolysis were less than 5 per cent (95–100 % recovery). Differences in duplicate analysis were less than 2 per cent.

DISCUSSION

At present, the only known function of chromium in human and animal nutrition is its involvement in carbohydrate metabolism. Although chromium is a functional part of GTF, only a little is known about the intestinal absorption and the biological availability of the element from foodstuffs. A true chromium deficiency has never been shown, whereas secondary deficiencies are known to occur, especially after parenteral nutrition (Jeejeebhoy et al., 1977). Studies done by Schroeder and others indicate that chromium levels in the tissues decrease with age (Schroeder, Balassa and Tipton, 1962; Schwartz and Mertz, 1959). Other studies conducted in the United States have shown that the impaired glucose tolerance in maturity-onset diabetes of elderly people im-

proved after oral administration of 2.89 μmol chromium (150 μg) daily as the inorganic salt (Meek, 1959).

The dietary requirement of chromium in man is estimated from balance studies. Most of the ingested chromium is excreted in the urine. Urinary excretion of chromium in young, healthy adults in the United States was found to be 0.19 $\mu\text{mol/day}$ (10 μg) (Mertz et al., 1978). Other losses from the skin and gastrointestinal tract have been estimated to be 0.10–0.19 $\mu\text{mol/day}$ (5–10 μg). Thus, Jeejeebhoy et al. (1977) suggested a maintenance intake of 0.39 $\mu\text{mol/day}$ (20 μg). It is known that the urinary excretion of chromium in subjects with an impaired glucose tolerance test is 2–3 times higher than in normal subjects, and as such, a higher dietary intake is recommended for diabetics, especially those who require insulin (Mertz et al., 1978). It is suggested that the fasting blood glucose in the age group above 60 years should not exceed 5.6 mmol/l (Scherstén, 1976); and on the basis of this recommendation, four subjects in the present study had fasting blood glucose levels that were too high. Results of the chromium intake showed that all

of the above four subjects had a daily chromium intake exceeding 0.77 μmol (40 μg). One subject who was being treated with chloralidone for hypertension had the highest chromium intake (11.3 μmol , 588 μg). It is difficult to interpret the significance of these results.

Biologically active chromium is different in different foodstuffs, and hence it is difficult to estimate optimum chromium requirements. The highest chromium contents are found in spices and certain herbs. Meat and vegetables are rather poor sources of chromium. According to Mertz et al. (1978), a safe chromium intake level is between 0.95 and 3.85 μmol per day (50–200 μg). One recent study in the United States showed a chromium intake of 0.10–2.21 μmol per day (5–115 μg) from three mixed diets (Levin et al., 1968). According to Schroeder (1970) the total daily intake of chromium in the United States is 4.7 μmol (245 μg). Our results are fairly close to this and we consider that the intake of chromium by the Dalby pensioners is adequate.

NICKEL

Recent studies by Nielsen (1971) have indicated that nickel is a dynamic trace element with a possible essential role in animals. Nickel is ubiquitous in nature and it is constantly found in many metabolically active tissues, such as the liver, kidney, thyroid and pancreas. It is also found in embryos. Schnegg and Kirchgessner (1978) have reported that growing rats fed on a nickel-deficient diet (0.3 nmol, 15 ng/g) had many metabolic malfunctions. They found that nickel deficiency interfered with iron absorption and resulted in anæmia. Moreover, they observed that several liver enzymes like ALAT, ASAT and LDH were affected by a nickel deficiency. Nickel is also known to activate several enzyme systems including arginase, acetyl CoA synthetase and trypsin

(Underwood, 1971). Currently, it is believed that nickel might be involved in the metabolism of membrane structures as well as in hormone control (Nielsen, 1976). Although the essentiality of nickel in human nutrition remains to be elucidated, there are indications that it might be of interest in the future (Nielsen, 1976).

MATERIAL AND METHODS

The same materials and technique as in the case of chromium analysis were used for the prep-

Table 20.II. The daily intake of nickel ($\mu\text{mol/day}$)

Men no.	Nickel	Women no.	Nickel
002	6.11	003	15.44
005	3.31	008	4.76
017	3.79	010	4.38
026	7.75	013	1.60
032	2.50	028	7.15
036	3.73	034	2.77
037	15.70	035	6.26
038	6.89	039	5.01
042	22.47	049	21.58
044	9.44	057	13.22
056	10.57	064	2.25
067	2.36	065	8.28
088	7.78	069	8.30
090	7.70	070	4.37
096	6.30	076	5.72
		080	2.68
		081	6.63
		082	7.53
		084	1.88
n = 15		n = 19	
Mean	7.8 (428 $\mu\text{g/day}$)	Mean	6.80 (400 $\mu\text{g/day}$)
Range	2.4–22.5 (139–1319 $\mu\text{g/day}$)	Range	1.6–21.6 (94–1283 $\mu\text{g/day}$)
Mean/1,000 kcal	3.96 (217 $\mu\text{g/day}$)		4.30 (253 $\mu\text{g/day}$)
		n = 34	
		Mean	7.3 (428 $\mu\text{g/day}$)
		Range	1.6–22.5 (94–1319 $\mu\text{g/day}$)

aration of samples prior to analysis by atomic absorption spectrophotometry. A recovery study was made by adding known amounts of nickel to the pooled dietary samples and following the normal procedure. A standard curve was made by using a nickel chloride solution (BDH Chemicals).

RESULTS

The complete results are shown in Table 20.II and Figure 20.2. The average intake of nickel was 7.8 μmol per day (428 μg), with a range of 2.4–22.5 μmol (139–1,319 μg) for the male pensioners. Corresponding values for the women were 6.80 μmol per day (400 μg) and 1.6–21.6 μmol (94–1,283 μg) respectively. The recovery study showed that the losses during hydrolysis were negligible (95% recovery).

DISCUSSION

Like chromium, nickel concentrations are very low in meat, milk and fruits. The highest con-

centrations are found in tea, coffee, cocoa, rice and vegetables. Tobacco contains rather high concentrations of nickel, and it is estimated that smoking 20 cigarettes a day will result in an accumulation of 3 mg of nickel in the lung yearly (Louria et al., 1972). According to Schroeder, the daily dietary intake of nickel in the United States is around 10.2 μmol per day (660 μg) (Schroeder, 1970). Drinking water in certain parts of the world, especially near nickel ores, may contain as high as 3.4 μmol (200 μg) nickel per litre (McNeely et al., 1972). Cans containing acidic fruit juices are another source of nickel contamination in commonly consumed foods. Fortunately, nickel is very poorly absorbed from the gastro-intestinal tract and as such does not present any serious health hazards. Toxic studies conducted in animals show that a concentration up to 17.0 mmol (1,000 mg) per kg dietary food intake does not produce any signs of toxicity (Pathak and Pathwardhan, 1952). The intake of nickel in the diet of pensioners in Dalby is slightly lower than the intake from the diet of the general population in the United States. The dietary intake levels in our subjects, however, were within safe limits.

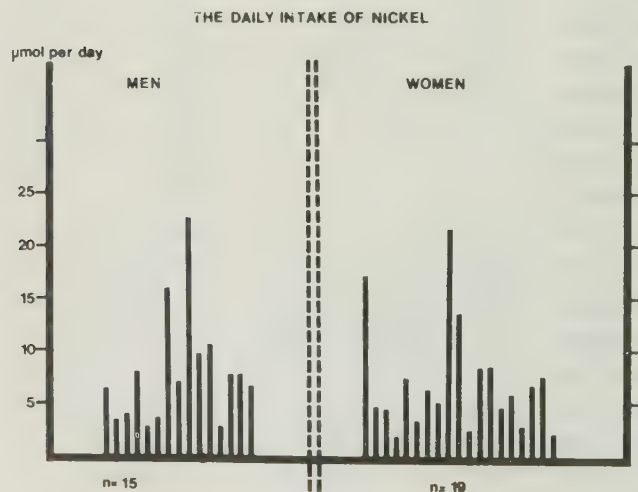


Figure 20.2. The daily intake of nickel by 34 Dalby pensioners.

Selenium

SELENIUM

MOHAMMED ABDULLA, KURT KOLÁR and SVEN SVENSSON

The essentiality of selenium in human nutrition is rather controversial at present. Initially, the biological role of selenium was confined only to its toxic effects on animals. Intensive studies conducted during the last 20 years have shown that selenium is essential for several animal species (Schwartz and Foltz, 1957; McCoy and Weswig, 1969; Thompson and Scott, 1970).

Rotruck et al. (1971) have recently shown that selenium is an essential constituent of erythrocyte glutathione peroxidase in several animal species. Another interesting aspect of selenium in animal nutrition is that it interacts with vitamin E (Jenkins and Hidioglou, 1972). According to Desai and Scott (1965), selenium exerts its effect by enhancing the retention of alpha-tocopherol through the formation of a selenium-containing plasma protein, which may transport the vitamin in the blood. Further, there are indications that selenium binds specifically to certain human plasma proteins (Burk, 1974). In heavy-metal poisoning, especially that caused by methyl mercury, selenium reduces the toxicity considerably (Ganther et al., 1972; Ueda, 1975).

As regards the chemical and physical characteristics of selenium, they are very similar to those of sulphur, but it is fairly clear that these two elements do not substitute for each other *in vivo* (Levander, 1976). The most recent studies by Schrauzer and others indicate that selenium has a vital role in the pathogenesis of several tumours in experimental animals (Schrauzer, 1976, 1978). Epidemiological studies that have been conducted in various

parts of the world suggest that the incidence of several malignant diseases is associated with a deficiency of selenium in the environment, especially in the drinking water (Schrauzer, 1978; TEMA 3, 1978). Certain areas of the world, such as Australia and New Zealand, have a very low soil selenium content, and this deficiency of selenium in soils is known to cause several disorders in a number of animal species (Underwood, 1971). The enrichment of some of these areas with selenium has resulted in a dramatic recovery by afflicted animals from selenium-deficient disorders (Underwood, 1971).

All of these factors have of late aroused interest in the role of selenium in human nutrition. Because there are no available data regarding the selenium content in the food of the general population in Sweden, a pilot study has been made to determine the selenium content in the diets of pensioners in Dalby, Sweden.

MATERIAL AND METHODS

The collection and preparation of the samples are described in Chapter 3. Seven daily portions, representing the different days of the week, were pooled to represent the average weekly dietary intake. Pooled 7-day samples from 11 women and nine men were included in the present study. The selenium content was analysed by a technique developed by Kolár and Widell (1977). A known amount of the finely homogenized fat-free food (usually 1–2 g) was wet-mineralized with concentrated nitric acid in

a special Teflon-coated stainless-steel pressure cooker at 160°C (160 atms. in the vessel). The mineralized contents were allowed to cool to room temperature and then were transferred to a volumetric flask. The residual nitrous gases were eliminated by heating with an ammonium oxalate solution. The cooled contents in the flask were then made up to a predetermined volume. The selenium content was analysed by atomic absorption spectrophotometry according to a method described by Manning (1971) after certain modifications (Kolár and Widell, 1977). Recovery studies showed that practically all the selenium added to the food samples could be recovered (85–103% recovery).

RESULTS

The complete results are shown in Table 21.I and Figure 21.1. The average intake of selenium from the total material was 0.39 µmol per day (30.8 µg), with a range of 0.11–1.22 µmol (8.7–96.3 µg) per day. The average intake of selenium in the women was 0.36 µmol (28.4 µg) per day, with a range of 0.11–1.15 µmol (8.7–90.8 µg). The corresponding

Table 21.I. The daily intake of selenium (µmol/day)

Men no.	Selenium	Women no.	Selenium
017	0.29	003	0.22
032	0.26	028	0.29
037	0.62	035	0.30
038	0.23	049	0.33
042	0.31	057	0.50
044	1.22	064	1.15
056	0.43	069	0.11
067	0.25	080	0.16
090	0.20	082	0.20
096	0.50	084	0.30
n = 10		n = 10	
Median	0.30 (23.69 µg/day)	Median	0.30 (23.69 µg/day)
Range	0.20–1.22 (15.79–96.33 µg/day)	Range	0.11–1.15 (8.69–90.80 µg/day)
Mean	0.43 (33.95 µg/day)	Mean	0.36 (28.43 µg/day)
Mean per 1,000 kcal	0.22 (17.2 µg/day)	Mean per 1,000 kcal	0.23 (18.0 µg/day)
n = 20		n = 20	
Median	0.30 (23.69 µg/day)	Median	0.30 (23.69 µg/day)
Range	0.11–1.22 (8.69–96.33 µg/day)	Range	0.11–1.22 (8.69–96.33 µg/day)
Mean	0.39 (30.79 µg/day)	Mean	0.39 (30.79 µg/day)

intake in the men was 0.43 µmol (34.0 µg), with a range of 0.20–1.22 µmol (15.8–96.3 µg) per day.

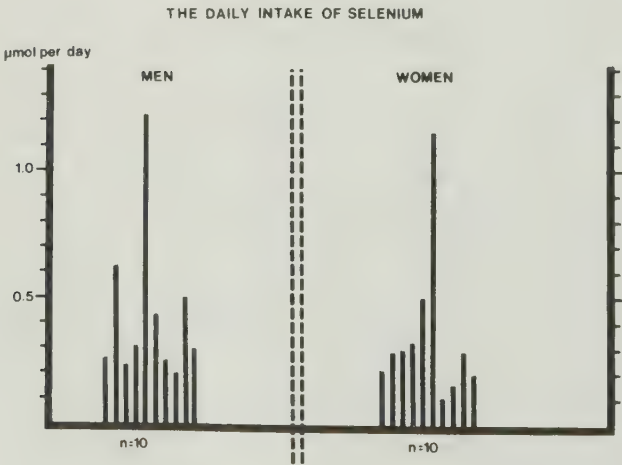


Figure 21.1. The average daily intake of selenium by 20 Dalby pensioners.

DISCUSSION

From a geobotanical and toxicological point of view of the known essential trace elements in animal nutrition, selenium is the least abundant in nature, but when it is found in excess, it is the most toxic micronutrient. At present, it is rather uncertain if selenium deficiency occurs in man. The selenium content varies considerably in different foods, as well as in food from different geographic areas. Australia, New Zealand, certain parts of North America and the Scandinavian countries are known to have comparatively smaller amounts of selenium in their soils, and accordingly a minimum supplementary intake of 30–100 μg (0.38–1.27 μmol) selenium has been recommended for several domestic animal species to prevent muscular dystrophies (Underwood, 1971). Because the selenium content in hay and grains from different parts of the Scandinavian countries is rather low, a working group within the Scandinavian Agricultural Research Association has recommended a minimum intake of 0.1 μg of selenium per gram (0.0013 μmol) fodder by the common domestic animals (Working Group 1975). No such recommendation is available at present regarding the dietary needs of selenium in man.

According to Schroeder, the selenium content in the daily diet of adult Americans is estimated to range between 0.76 and 1.90 μmol (60–150 μg) (Schroeder et al., 1970). Further, Schroeder and co-workers have found a selenium content of 329 nmol (31 μg) in hospital diets (Schroeder et al., 1970). In New Zealand, where the selenium content is known to be extremely low in the soil, Griffiths found an intake of 8–886 nmol (6–70 μg) selenium per day in the diet of 13 women (Griffiths, 1973). In some parts of Canada where the soil is rich in selenium, Thompson et al. (1975) found a daily intake of 2,494 nmol (197 μg) selenium, with a

range of 1,430–2,785 nmol (113–220 μg). According to the published literature, kidney, liver, various meat products and several varieties of corn are good sources of selenium, whereas vegetables and fruits are rather poor sources in comparison. A recent study by Kolár and Widell (1977) showed considerably lesser amounts of selenium in the liver, kidney and muscle of domestic animals in Sweden than in other parts of the world. This might be due to the low selenium content in Swedish soils. Studies conducted by Higgs et al. (1972) indicated that cooking may reduce the amount of biologically active selenium in some foods, especially in vegetables. It is likely that people living on a pure vegetarian diet, especially if the food is consumed after cooking, may risk a low intake of selenium.

The major excretory pathway of selenium is the urine, and Griffiths (1973) has recently found a good correlation between selenium intake and excretion in the urine. Nearly 50 per cent of the dietary intake of selenium has been found to be excreted in the urine (Griffiths, 1973). Further, it has been found that the selenium content in 24-hour urine samples is significantly correlated with the selenium levels in the blood. Because the selenium content of the blood is generally very low (0.72–1.18 μmol , 57–93 $\mu\text{g/l}$), it is difficult to measure, and the results that are obtained are often unreliable. Measurements of erythrocyte glutathione peroxidase activity may give a good indication of the selenium level in blood (Ganther et al., 1976).

A very interesting and important aspect of selenium in human nutrition is its probable role in the prevention of malignant diseases. Recent experimental studies have indicated that selenium has cancer-protecting properties in animals (Schrauzer, 1976, 1978; Harr et al., 1972). Additions of subtoxic amounts of this element as selenite in the drinking-water

lowered the incidence of spontaneous mammary tumours in mice (Schrauzer, 1978). Epidemiological studies conducted in various parts of the world suggest that human cancer mor-

tality is lower in areas rich in selenium (Schrauzer, 1978; TEMA 3, 1978). These aspects need further documentation.

Iodine

IODINE

MOHAMMED ABDULLA, MARGARETHA JÄGERSTAD, ARNE MELANDER, ÅKE NORDÉN,
SVEN SVENSSON and ELISABETH WÄHLIN

Iodine is known to be an essential part of the calorogenic thyroid hormones. The thyroid gland contains 10-15 mg of iodine. However, also the salivary glands, the mammary glands, the placenta and a few gastro-intestinal glands store small amounts of iodine (Ganong, 1973). The total body content of iodine amounts to approximately 50 mg, but the physiological significance of the extrathyroidal stores is not known (Ganong, 1973; Harper, 1975).

The average daily Western diet comprises about 100-400 µg of iodine, mainly in the form of iodide. The major part is absorbed from the small intestine and about 70-90 per cent of the iodide is excreted by the kidney (Harper, 1975). A deficient iodine intake promotes goitre formation and, if severe enough, hypothyroidism.

During the last decade, several measures have been taken to increase the dietary level of iodine in Sweden. In 1966, the iodization of table salt was increased to 50 mg/kg, either in the form of potassium or sodium iodide. Apart from table salt, sea foods, milk and dairy products are the most important dietary iodine sources. During 1971, an iodine fortification of the mineral feeds given to dairy cattle was introduced, and it was followed by a significant rise of the iodine content of milk (Iwarsson, 1974). In addition, the introduction of iodophor-dipping for teat disinfection may unintentionally lead to increased iodine concentrations in milk (Iwarsson and Ekman, 1974).

The double portion sampling technique

makes it possible to determine the daily intake of iodine using chemical analysis. The methods for iodine determination have been improved regarding both extraction procedures and sensitivity in recent years. To assess the accuracy of the chemical method used in the present study, some of the food samples were also analysed with the neutron-activation method.

MATERIAL AND METHODS

Determination of dietary iodine

A. Chemical analysis

Iodine was determined in fat-free lyophilized food homogenate pooled from seven 24-hour diets representing all the days of a week (see Chapter 3). All chemicals used were of analytical grade. Glassware was soaked in 30 per cent nitric acid and rinsed several times with deionized water and glass-bi-distilled water before use. Glass-bi-distilled water was also used as a diluent.

Food powder (100 mg) was digested in concentrated acids (3.5 ml sulphuric acid, 0.1 ml nitric acid and 0.4 ml 70 per cent perchloric acid) according to Malvano et al. (1972). The wet-ashing procedure was performed on a sand bath with a surface temperature between 240 and 260°C for 30 minutes. Thereafter, gaseous nitrogen was bubbled through the samples until they became colourless, generally within 10

seconds. After cooling at room temperature, the samples were diluted with water to 10 ml. Blanks and recovery samples were always included.

The chemical determination of iodine was based on the Sandell-Kotloff reaction. The analysis was performed as described by Pauwells and Wesemael (1962). Each sample was wet-ashed and analysed on two different occasions. Two millilitres of each sample were used in the assay. The iodine content was calculated from a standard curve prepared as follows: An iodine standard curve was wet-ashed together with 100 mg of lyophilized and recrystallized egg albumin (grade III, Sigma), viz. analogous to the preparation of food samples. The egg albumin contained no iodine. A standard curve prepared after wet-ashing compared with one directly diluted is shown in Figure 22.1. Using the radio-isotope ^{125}I (as KI) the recovery was calculated to be 82 per cent. The mean and S.E.M. of eight determinations of one

food sample on different occasions was 1.30 ± 0.07 .

B. Neutron-activation analysis

In order to assess the accuracy of the chemical method, 10 food samples were analysed by neutron activation. The analyses were run in duplicate by AB Atomenergi, Studsvik, Sweden. The recovery was 82–85 per cent. In Table 22.I ten food samples analysed with both methods are shown.

Determination of thyroid hormones

The total serum concentrations of thyroxine (T_4) and 3,5,3-tri-iodothyronine (T_3) were measured on samples obtained at about 8 a.m. in the fasting state. The determinations were carried out by radio-immunoassays, as devised by Burger (1974) and Burger et al. (1975).

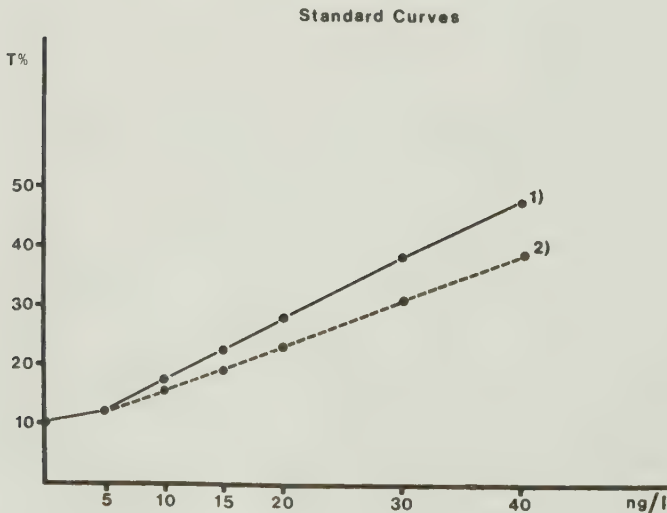


Figure 22.1. ¹The standard curve, wet-ashed together with iodine-free egg albumin.
²Directly diluted standard curve.

Table 22.I. Comparison between food samples analysed with a chemical method and by neutron activation

Subject no.	Chemical analysis (µmol/day)	Neutron activation (µmol/day)
03	0.65	0.61
05	1.50	2.12
08	1.37	1.27
10	1.40	1.50
26	1.96	0.52
44	1.81	1.51
45	3.54	2.32
56	2.35	1.34
64	2.28	1.74
84	1.76	1.14
Mean	1.86	Mean 1.41
S.D.	± 0.77	S.D. ± 0.58

RESULTS

Dietary intake of iodine

The average daily intake of iodine is shown in Figure 22.2 and Table 22.II for both men and women. The mean intake by the 16 male pensioners was $2.51 \pm 1.0 \mu\text{mol}$ ($318 \pm 127 \mu\text{g}$). The 19 female pensioners consumed an average of $1.94 \pm 0.83 \mu\text{mol}$ ($246 \pm 105 \mu\text{g}$). The range for the male group was 0.63-4.53 μmol (81-575 μg), and for the female group 0.65-4.06 μmol (82-516 μg). When expressed per 1,000 kcal (4.2 MJ), both the male and female pensioners consumed, on an average, 1.2 μmol (160 μg).

Serum concentrations of T_3 and T_4

The total serum concentrations of T_3 and T_4 are shown in Table 22.III. The mean ($\pm$ S.E.M.) concentration of T_3 was $2.70 (\pm 0.01) \text{ nmol/l}$ in the male group (range 1.76-3.26), and $2.69 (\pm 0.08) \text{ nmol/l}$ in the female group (range 2.34-3.25). The corresponding T_4 mean values

were $110 (\pm 6)$ and $124 (\pm 5) \text{ nmol/l}$, and the ranges 66-161 nmol/l and 80-160 nmol/l for men and women, respectively.

DISCUSSION

Dalby is located in a maritime area within a province where endemic goitre has never been prevalent. The most abundant dietary iodine sources are table salt, salt-water fish and milk products. Fortification of table salt was first introduced in 1930, and the amount was further increased in 1966. As calculated in Chapter 13, the male and female pensioners consumed an

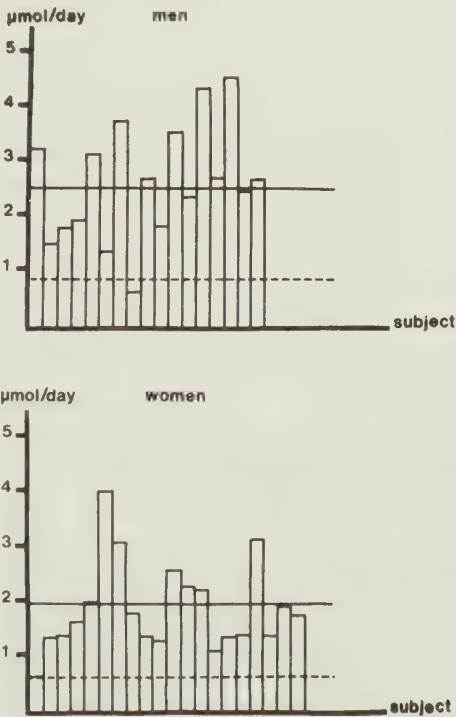


Figure 22.2. The mean daily intake of iodine, expressed in μmol , by 16 male and 19 female pensioners. The unbroken line indicates the mean level for the respective groups. The broken line is the intake estimated to meet the daily requirements according to *Recommended Dietary Allowances* (1974).

Table 22.II. The mean daily intake of iodine determined in pooled 7-day diets

Men subj. no.	µmol/day	Women subj. no.	µmol/day
02	3.22	03	0.65
05	1.50	08	1.37
17	1.80	10	1.40
26	1.96	13	1.67
32	3.16	28	2.02
36	1.35	34	4.06
37	3.77	35	3.10
38	0.63	39	1.81
42	2.69	49	1.41
44	1.82	54 ¹	1.30
45	3.54	57	2.62
56	2.35	64	2.28
60 ¹	4.36	65	2.26
67	2.68	69	1.10
88	4.53	70	1.41
90	2.42	76	1.42
96	2.67	80	3.17
		81	1.41
		82	1.93
		84	1.76
Mean	2.50 (318 µg)		1.94 (246 µg)
S.D.	1.0 (127 µg)		0.83 (105 µg)
Range	0.65-4.53 (81-575 µg)		0.63-4.53 (82-516 µg)
n	16		19

¹ Excluded from the calculation of mean, S.D. and range for the whole group.

average of 100 and 78 nmol of sodium per day, respectively. This corresponds to a daily intake of about 4.5-6.0 g of sodium salt. If all of this sodium originated from iodized sodium chloride, about 200 µg iodine per day should be obtained from this source. In Figure 22.3, the correlation between the intake of sodium and iodine is shown. There was a significant correlation between the intake of sodium and iodine for the men ($p < 0.05$), but surprisingly not for the women ($p > 0.25$).

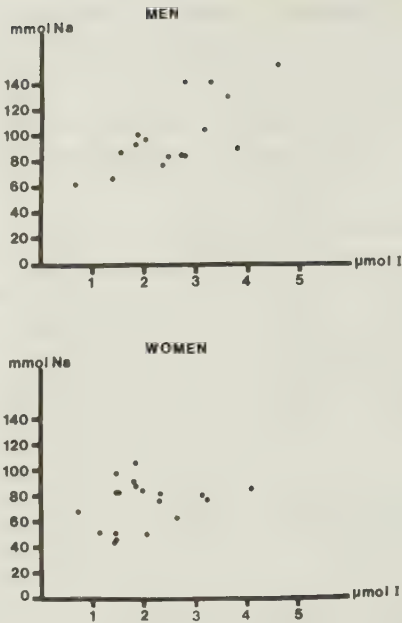


Figure 22.3. The correlation between the intake of sodium and iodine. The correlation coefficient was 0.69 ($p < 0.05$) and 0.21 ($p > 0.25$) for male and female pensioners, respectively, using non-parametric rank correlation test.

Milk is estimated to provide 16 per cent of the dietary iodine. After the introduction of iodized mineral feeds for dairy cattle, milk has contained about 50-100 µg/l (Iwarsson, 1974). The milk consumption by the pensioners can be calculated from data of the lactose intake (see Chapter 11). Because milk contains 5 per cent lactose, the average daily intake of milk would be 350 and 250 ml for the men and women, respectively. This would yield about 20-30 µg iodine.

The third important iodine source is salt-water fish. According to interviews, fish is relatively often consumed by the pensioners in the present study, and it may thus have contributed to the high iodine intake in several cases.

According to the Food and Nutritional Board (1970), the daily iodine requirement in adults is estimated to be 50-75 µg (0.4-0.6 µmol), or approximately 1 µg/kg body-weight.

Table 22.III. Serum concentrations of T₃ and T₄, expressed as nmol/litre

Men			Women		
Subject no.	T ₃	T ₄	Subject no.	T ₃	T ₄
2	2.66	126	8	3.14	160
5	3.26	161	10	2.36	116
17	2.74	125	13	2.77	142
26	3.05	119	34	3.20	106
32	2.86	107	35	2.48	115
36	2.20	101	39	2.65	144
37	2.91	110	49	2.56	126
38	2.73	106	54	2.31	157
44	2.97	95	57	2.94	116
45	2.57	106	64	2.34	119
56	1.76	92	65	2.93	120
88	2.97	112	69	2.73	134
90	1.14	51	76	2.11	112
96	2.47	90	80	2.49	80
			81	2.77	99
			82	2.74	154
			84	3.25	111
Mean	2.59	107		2.69	124
S.D.	0.54	23		0.32	21
Range	1.14-3.26	51-161		2.11-3.25	80-160
n	14	14		17	17

Furthermore, an intake between 50 and 1,000 µg of iodine (0.4-8.0 µmol) by adults has been deemed to be safe, and intakes between 100 and 300 µg (0.8-2.4 µmol) are desirable. In ages above 51 years, a daily intake of 110 µg (0.9 µmol) for men and 80 µg (0.65 µmol) for women is recommended, according to *Recommended Dietary Allowances* (1974).

The intake of iodine recorded in the present material is considerably above the recommended minimal amounts. Indeed, the mean intake is greater than the highest figure hitherto reported for a Swedish population, namely, 133 µg/day. This was recorded for people living in a sea-coast area (Abramson, 1961). The lowest intake observed was recorded in subjects living in the interior parts of Sweden. They had an es-

timated consumption of only 22 µg/day (Abramson, 1961).

In a survey based on calculations of the 24-hour urinary excretion of iodine of subjects in an area where endemic goitre has been frequent, Järnerot and Karlberg (1974) reported that 10 per cent of the male subjects and 33 per cent of the female subjects excreted less than 70 µg per day in the urine. Because 70-90 per cent of the dietary iodine is excreted in the urine, they concluded that the intake of iodine was inadequate in these subjects. However, in a more recent study, also carried out within a previously goitre-rich area and employing the iodine-excretion technique, Gustavsson et al. (1977) obtained evidence that the iodine intake during later years has increased to sufficient

amounts, probably due to the overall use of iodized salt. Thus, it seems probable that the current intake of iodine is adequate from the goitre-preventive point of view.

The finding of a large average intake of iodine in the present study accords with the observation that each subject in the present material had T_3 and T_4 values well above the lower nor-

mal limits at our laboratory (1.5 nmol/l and 65 nmol/l, respectively). Indeed, the mean values were close to the upper normal limits (3.2 nmol/l and 155 nmol/l, respectively). Obviously, the iodine intake in the population studied suffices to maintain a normal thyroid function and may even be unnecessarily large.

***Essential and toxic inorganic elements
in prepared meals - 24-hour dietary
sampling employing duplicate
portion technique***

ESSENTIAL AND TOXIC INORGANIC ELEMENTS IN PREPARED MEALS -
24-HOUR DIETARY SAMPLING EMPLOYING THE DUPLICATE PORTION TECHNIQUE

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Introduction

Rapid improvements in analytical technology and sophisticated instrumentation during the last few decades have helped to disclose the presence of most of the naturally occurring chemical elements in living systems and their food chains. Recent estimates indicate that most tissues of a healthy human adult contain as many as 40-80 elements represented in the periodic table of the elements (1, 2). In studies concerning the health and diseases of man, the inorganic chemical elements are classified according to their essential and non-essential role in biochemical processes. At present, at least 21 elements represented in the periodic table have been found to be absolutely essential for human life (3). This number is likely to be increased in the future with improvements in our knowledge concerning the role of the elements in vital metabolic reactions. Of the 21 elements that are known to be essential, the so-called trace elements, such as iron, zinc, copper, manganese, chromium and selenium, have attracted considerable attention in nutritional research during the last few years. In spite of the tremendous progress that has been made in the field of trace element nutrition research in the past, the biological role and the minimum requirement of many elements are still hypothetical. The minimum requirement of some of the elements that have been found to be essential in human nutrition is so low that it is generally believed that a pure nutritional deficiency of these elements rarely occurs in man. During the past few decades, rapid developments in food technology have

enabled the growing food industry to offer the general public in industrialized countries an enormous choice of food products. The increased consumption of highly refined food stuffs for extended periods may result in marginal deficiencies of several trace elements (4). At present there are no techniques available for diagnosing subclinical deficiencies of trace elements at an early stage. There are indications at present that marginal deficiencies of trace elements such as zinc, copper and selenium do occur in the general population of developed countries (5). Certain groups including children, pregnant women and the elderly may be more vulnerable to such deficiencies than the population in general.

Except for occupational exposure, the major pathway through which chemical elements enter the human body is through the food chain. Although most of the elements in individual food items can be accurately measured today, there is no reliable data for the minimum requirement of several important elements. One reason for this situation is due to the lack of information concerning the dietary intake of the elements from prepared meals for human consumption. The data that is normally reported in the literature concerning the dietary intake of major nutrients is very often based on diet surveys conducted in different parts of the world employing conventional techniques like diet history and interview studies in combination with food tables. These methods have severe limitations when used for the assessment of trace element intakes. The intake levels of elements like copper, chromium, manganese and selenium are difficult to compute from food tables because of their low concentration in the diets. Moreover, most of the food tables give only average values even for the bulk elements such as calcium, magnesium, sodium and potassium. Such values are calculated without taking into consideration the various factors such as processing and cooking which alter the concentration of many nutrients prior to actual consumption. Trace elements in individual food items may also vary in different parts of the world due to seasonal changes and the differences in geographic distribution of the elements in the soil. Very often, many food tables have no information at all concerning the concentration of several important essential trace elements. The present study describes the technique developed in our institute for the

assessment of the dietary intake of essential and toxic elements from prepared meals in various groups of the general population in Sweden.

Material and Methods

The 24-hour dietary samples were collected employing the duplicate portion technique. (A duplicate portion is an exact copy.) It represents, then, as exactly as possible, the food and liquids--collected by visual estimation--consumed during a 24-hour period (Fig. 1). The material collected during the day was subsequently pooled in a single container. Due care was taken to omit food not actually consumed. The subjects participating in our studies were thoroughly briefed by a trained dietician prior to the initiation of the study regarding the proper method of collecting the duplicate portion food samples. On the day of food sampling, a written protocol was also kept that described the type and approximate quantities of the various food items consumed. The pooled material was later transported to the laboratory. Here it was weighed and homogenized. A suitable aliquot of the homogenized material was then lyophilized. The lyophilized powder was later subjected to fat extraction using chloroform and methanol for the analysis of fat and fat-soluble components of the diet (6). The fat-free powder was dried and quantitated. This powder was used for analyses of most inorganic chemical elements. Approximately 0.5 g of the fat-free powder was wet ashed using concentrated sulphuric, perchloric and nitric acids. The temperature during the wet-ashing procedure was kept under 250°C. A typical wet-ashing procedure took 6-8 hours. Normally 8-12 samples were wet-ashed per day. Each series of dietary samples contained at least one blank containing the proportions of acids only and treated in the same way as the dietary samples. Recovery studies were performed by adding known amounts of standard solutions containing the metals to be analysed. The complete procedure is described elsewhere (7). For the determination of cadmium and lead in the diets, 15 g of the fresh homogenate was used and the metals were extracted with methylisobutylketone (MIBK) after wet-ashing (7). The mercury contents were determined by ashing 0.3 g of the fat-free powder in an atmosphere

of oxygen according to a method described by Schütz (8). A few 24-hour samples were also subjected to neutron activation for the comparison of the results with that obtained by other techniques.

Food samples were collected from 20 normal adults of working age (25-60 years old, 10 men and 10 women), 60 women (30-79 years old) who were involved in an ophtalmological survey (9), 6 vegans (49-55 years old) and 6 lacto-vegetarians (49-52 years old). The first group of 20 adults collected 7 daily dietary portions, each representing seven different days of the week. Similarly, the pensioners (17 men and 20 women) collected 259 dietary portions. The 60 women collected 6 daily dietary samples each and the vegans and lacto-vegetarians 4 samples each. Thus, a total of 807 daily dietary portions were collected. In the cases of the 60 women and the vegetarians, we also collected 24-hour urinary samples corresponding to the days of food sampling. This was done mainly to measure the excretion of electrolytes and nitrogen.

Results

The representativeness and reproducibility of the lyophilization and extraction procedures were checked by analysing different aliquots of the homogenates on different occasions. The correlation between the dry weights of the fat-free and water-free substances obtained on two different occasions was excellent. The correlation coefficient was 0.999 and the variation coefficient 0.81 per cent. We also investigated the losses of the chemical elements that occurred during the lyophilization and fat-extraction procedures by analysing the homogenates directly and comparing the results with those obtained from the fat-free powder. In most cases the losses were less than 5% (7). Recovery studies showed that the wet-ashing procedure resulted in a loss of 10% as regards sodium and potassium. For the remaining elements investigated in the present study, the losses due to the wet-ashing procedure were less than 5%. The difference between duplicate determinations in most cases was within ± 2.5 per cent.

Figure 2 shows the summary of results concerning the concentration of sodium, potassium, calcium, magnesium, iron, zinc, copper and selenium. Although there are no internationally accepted standards for the optimal intake of many elements, we have shown in the figure the range of the recommended dietary allowance that is usually quoted in the literature. As can be observed, the concentration of most of the elements in non-vegetarian diets is low when compared with the recommended range. This is especially germane for potassium, magnesium, zinc, copper and selenium. Vegetarian diets, on the other hand, had much higher concentrations of these elements when compared with the non-vegetarian diets. Selenium content in the vegan diets was extremely low. The lacto-vegetarian diets, on the other hand, had a higher concentration of selenium than the other diets. Table 1 shows the concentration of lead, mercury, cadmium, nickel, chromium and iodine in pensioners' diets. The concentrations of heavy metals in the vegetarian diets have not yet been analysed. The contents of mercury, cadmium and lead in pensioners' diets are very low when compared with the reported values from other European countries and the United States. The concentrations of nickel, chromium and iodine found in the diets, on the other hand, are very similar to the values reported from other industrialized countries. When compared with the present recommendations, they are far more than adequate. The iodine and selenium contents analysed by neutron activation analysis were lower (10-15%) than that is reported here.

Figures 3 and 4 show the correlations between the data obtained through the food tables and those from chemical analyses of iron and calcium in the hospital diets. The computed data in general give higher values than those that are obtained through chemical analyses. The computed data could not be used for the comparison of most trace elements because there are no values given for them in the food tables.



Fig. 1. Illustration of the procedure for collecting a duplicate portion from a standard hospital meal.

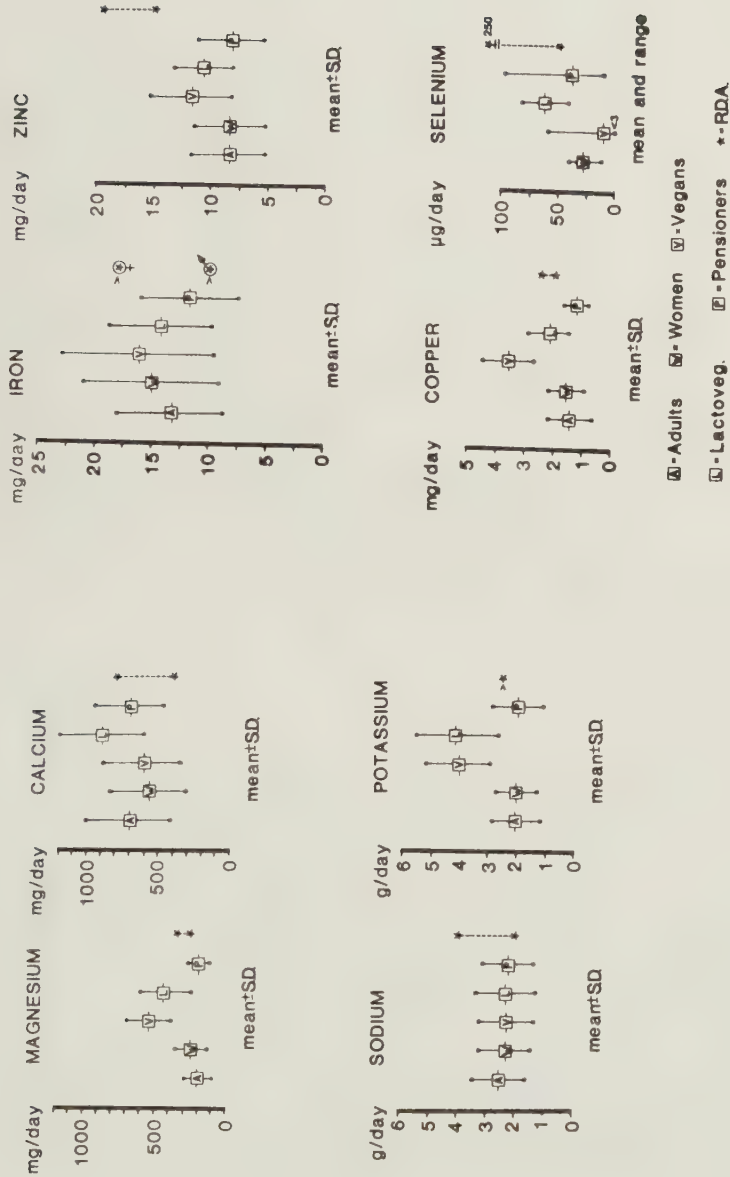


Fig. 2a

Fig. 2b

Concentration of essential elements in the duplicate portions of healthy adults, pensioners, women, vegans and lactovegetarians together with the range of RDA levels.

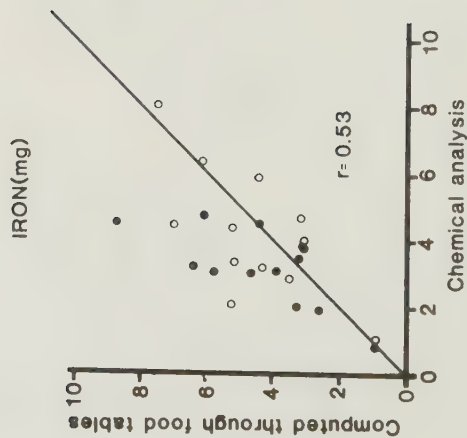


Fig. 3. Comparison of iron concentrations in hospital diets computed through food tables and by chemical analysis

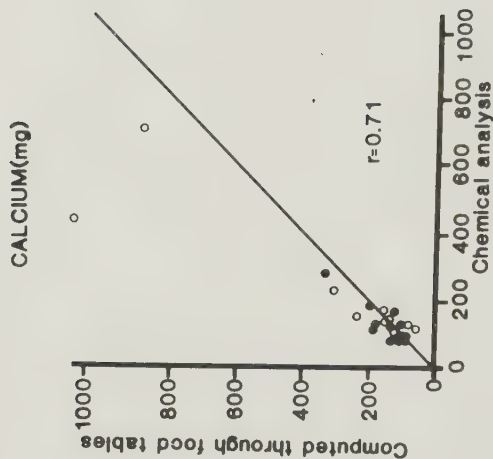


Fig. 4. Comparison of calcium contents in hospital diets computed through food tables and by chemical analysis.

TABLE 1. Concentration of nickel, chromium, iodine, lead, cadmium and mercury in 24-hour dietary portions of pensioners.

Element	No. of portions	Concentration $\mu\text{g/day}$ mean and range
Nickel	34	428 (94-1319)
Chromium	34	182 (44-588)
Iodine	35	282 (81-575)
Lead	72	36 (6-86)
Cadmium	49	12 (4-35)
Mercury	53	4 (0.6-19.5)

Discussion

Although indispensable for mass production and mass surveillance of food, the tables showing the composition of individual food stuffs may not necessarily provide a true picture of what the individual is actually eating. The food tables that are generally used for assessing food composition are based on data obtained from chemical analyses of raw food materials. Such data may not apply to mixed diets served on the plate for consumption. The changes that may occur in the raw food stuffs during processing and cooking are very important to consider. Further, the food tables seldom have information regarding new food products owing to the lack of analytical techniques for determining the individual components. Development of the duplicate portion technique has eliminated many of these problems. For the first time, we have been able to provide accurate information, concerning the dietary content of many essential and toxic elements that have previously been difficult to determine. Another advantage of this technique

is the possibility to store the dietary samples for future use. According to the current trend, the number of elements that may be essential for human nutrition is likely to increase in the future. With current trends in the sophistication of analytical instrumentation, one can practically analyse all the naturally occurring chemical elements in biological materials and food samples in the near future. We have plans at this time to store our dietary samples for a considerable number of years in the frozen state. Thus, it will be possible to analyse, at any time in the future, a particular element of interest that had been omitted in the original investigation. This is important in view that subclinical deficiencies of several essential trace elements may develop after many years.

One of the major criticisms that has been directed towards the present technique concerns the possible bias that may be introduced during sampling. Do the participants collect true copies of what they normally eat? This aspect has been investigated in detail in the study of the 60 women. In this group the urinary volume and the electrolyte excretion were determined on two occasions without simultaneous collection of dietary samples. On the third occasion these 60 women collected 6 dietary samples, and 3, 24-hour urinary samples corresponding to the days of food samplings. Once again the urine volume, concentration of sodium, potassium and nitrogen were determined. The results indicated no significant differences between the three investigations. Thus, the food collection did not disturb, in any way, the dietary habits of those taking part in our studies.

Another point of interest involves the low values obtained by the present technique as compared with the computed data, especially as regards total energy intake. One reason for this difference can be attributed to the differences in the analytical procedures. The results as regards the contents of essential elements in different series of dietary samples in our laboratory and in studies by other investigators are, however, reproducible. Also, another way of controlling the validity of our data is by measuring the energy intake and expenditure by an indirect technique. Such studies are, however, time-consuming and complicated.

As can be observed from the results, the contents of most essential elements investigated in the present study were low when compared with the recommended dietary allowances (RDA). Similar results have been found in the United States. Over-consumption of highly refined foods may be one of the reasons for this low intakes of essential elements. This can be easily seen from the results of the vegetarian diets. The contents of potassium, magnesium, copper and zinc in the vegetarian diets were particular high. All of these elements have been found to be low in normal diets. The pensioners participating in the present study have received regular medical examinations and the clinical parameters after a 6-year follow-up indicated no signs of deficiency of any nutrients. As pointed out earlier, it is difficult to detect subclinical deficiencies of most essential elements. Measurements of serum levels of the elements alone may be inadequate for assessing body status. There is an urgent need for sensitive parameters for detection of the intracellular status of trace elements such as zinc, copper and magnesium.

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***Comparison of energy and nutrient
intakes in women with high and
low blood pressure levels***

Comparison of Energy and Nutrient Intakes in Women with High and Low Blood Pressure Levels

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ABSTRACT. The present study found no link between the intake of energy and various nutrients, on the one hand, and high or low blood pressure (BP) in women, on the other. Sixty women not on treatment for hypertension were selected from a defined population and examined, applying the duplicate portion technique, with respect to the relationships between BP and the intake of energy and nutrients. They were selected from above the 95th percentile for BP (group A) and from below the 30th (group B). The two groups were age-matched. The food sampling comprised six days, divided into three periods of two consecutive days within a period of four weeks. Twenty-four-hour urine specimens were collected in each period and on two other occasions. The mean values for intake of energy, fat, protein, carbohydrates, minerals and electrolytes did not differ between the two groups despite the large differences in BP and obesity. The mean values for urinary excretion of minerals, electrolytes and nitrogen (calculated as crude protein) did not differ between the groups. The present findings for the effect of salt on BP do not justify restriction of the salt intake as a means for decreasing BP in the population.

Key words: duplicate portion technique, energy intake, excretion of minerals and electrolytes, high and low blood pressure levels, population study, salt intake, women.

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Obesity and overweight are important risk factors for hypertension (6, 13, 23) and there is evidence that a decrease in body weight usually leads to a reduced blood pressure (BP) (12, 29). Therefore, weight reduction should be of value in the treatment of hypertension. However, it is common experience that weight reduction is difficult not only in the extremely obese but even in the large number of persons who are moderately overweight. Another

risk factor is excessive salt intake (8, 21, 31). Accordingly, a reduced consumption of salt may promote decreased BP (27) and moderate salt restriction has been used either as the sole treatment of mild hypertension (26) or in combination with diuretics (28). Owing to the results of these studies, reduced salt consumption as a dietary goal has won official approval in the USA (20).

The present investigation deals with the question whether subjects with high and low BP differ in their dietary habits, especially with respect to their intake of energy and salt. The investigation was carried out with the duplicate portion technique (3, 4).

SUBJECTS

The subjects were selected from participants in an ophthalmological survey performed in the Dalby population during 1969–70. In that study BP was measured in a strictly standardized fashion as described previously (34). The survey comprised 963 females, of whom 790 (82%) completed the study. All the latter were without antihypertensive treatment at the time of selection. From this female population, those with BP above the 95th percentile (group A, $N=36$) in the age range 30–79 years were selected and age-matched with 36 females selected from those with BP below the 30th percentile (group B). The subjects in group A were selected on the basis of systolic and diastolic BP and pulse pressure; they were included if any of these indices or any combination thereof exceeded the 95th percentile in the whole material for the five age groups 30–39, 40–49, 50–59, 60–69 and 70–79 years. Subjects were assigned to group B if both the systolic and the diastolic BP were below the 30th percentile. The pulse

Abbreviations: T_3 = triiodothyronine, T_4 = thyroxine, BP = blood pressure.

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pressure was not used as a selection criterion for group B in order to avoid including females in whom low pulse pressure was combined with elevated diastolic BP.

In group A, one subject refused to participate, while another failed to attend all investigations. A third was on antihypertensive treatment and a fourth had developed diabetes mellitus after the first examination. These four subjects and their matched controls in group B were excluded. Furthermore, one female from each group failed to manage food sampling by the duplicate portion technique. Hence, these two subjects and their matched controls were also excluded and each group finally comprised 30 females. In 1975, the age in group A was 52 ± 13 and in group B 52 ± 14 years (mean $\pm$ S.D.).

METHODS

Anthropometric and BP measurements

Height and weight were measured according to the WHO recommendations (30). Relative body weight was calculated as weight (kg)/height (cm)–100 and the index of obesity as weight (kg)/height³ (m) (36).

The technique for BP measurement has been described in detail (33).

Biochemical variables

The analyses of B-hematocrit, fB-glucose, fS-cholesterol, fS-triglycerides, and S-urate levels were performed by conventional techniques. The analyses of S-triiodothyronine (T₃) and S-thyroxine (T₄) were carried out by selective radioimmunoassays (5, 25).

Sampling of food and urine

The dietary habits were investigated during Jan.–May, 1975. The duplicate portion technique (3, 4) was used to study the intake of energy, fat, protein, carbohydrates, and minerals. Participants were informed about the investigation and the duplicate portion technique, and given precise instructions for urine collections. The food sampling was carried out within a period of four weeks. It comprised six days divided into three periods of two consecutive days, Monday–Tuesday, Wednesday–Thursday and Saturday–Sunday. During the second day in each of the three periods, 24-hour urine specimens were collected (referred to as 1975:2). The participants also made up written protocols—giving not only the type of food eaten but also crude estimates of the quantities expressed in colloquial terms—for each of the sampling days, in order to trace the source of unexpected values.

Analyses of nutrient and energy intake

The duplicate portions were kept in the refrigerator overnight and then brought to the laboratory. After weighing, the portion was homogenized, lyophilized, and fat-extracted with chloroform as described earlier (3, 4). In the present study, however, sodium desoxycholate as emulsifier was changed to Triton® X-100 (isooctyl-phenoxypolyethoxy-ethanol, Kebo-Grave, Sweden), in order to avoid sodium contamination. Total fat content was obtained after evaporation of the chloroform extract. The

protein content was determined as nitrogen, using a slightly modified Kjeldahl method (18, 24). Nitrogen ($\text{g} \times 6.25$) gave the crude protein intake. The carbohydrate amount was obtained "by difference": g fat-free powder minus g protein and 7% ash. The energy intake was calculated from g fat $\times 9.3$ plus protein $\times 4.1$ plus g carbohydrate "by difference" $\times 4.1$ (3, 4).

Minerals (calcium, magnesium, zinc, copper) were analysed by atomic absorption spectrophotometry after wet-ashing from the fat-free lyophilized food homogenates (4). Sodium and potassium were determined by flame photometry as described earlier (4). Analyses of nitrogen, minerals and electrolytes in the urine samples were performed in order to compare the dietary intake and urinary excretion of these substances.

Furthermore, the 24-hour urinary excretions of sodium and potassium (1975:2) were compared with the corresponding results from two other 24-hour urinary collections in the two groups (1972 and 1975:1). In group B, the 24-hour urinary excretion of nitrogen was also analysed in 1972 and compared with the corresponding results in 1975:2.

Statistical methods

Mean values, standard deviations, coefficients of variation and correlation coefficients were determined by conventional methods. Most calculations were performed at the Computer Centre, University of Lund, Lund. Statistical analyses were also made with an Olivetti Programma 101. Statistical significance was generally calculated by Student's *t*-test on paired or unpaired data. The validity of the urinary collections was determined by analyses of variance for sodium and potassium excretions on the five occasions (one 1972, one 1975:1 and three 1975:2). The significance levels are expressed as follows: $0.01 < p < 0.05$, $0.001 < p < 0.01$, $p < 0.001$. The following symbols are used: *N*=number, *M*=mean, S.D.=standard deviation, DF=degree of freedom, n.s.=not statistically significant ($p > 0.05$), *r*=correlation coefficient. All values are given as $M \pm \text{S.D.}$ unless otherwise stated.

RESULTS

Anthropometric measurements

The results in 1972 and 1975 are given in Table I. The mean values of weight, relative body weight and the index of obesity were all higher ($p < 0.001$) in group A than in group B. There was no significant difference in height. All these values remained essentially unchanged between 1972 and 1975.

Blood pressure

The BPs in groups A and B at the first study and at the follow-up examinations are given in Table II. The differences in systolic and diastolic BP between the two groups persisted from the time of selection in 1969–70 to the follow-up examinations in 1972 and 1975. In 1972, mean systolic BP was higher than

Table I. Anthropometric measurements in 1972 and 1975

	Group A	Group B	<i>p</i>
1972			
Height (cm)	161±6	163±6	n.s.
Weight (kg)	76.1±15.7	59.7±10.0	***
Relative body weight	1.24±0.27	0.96±0.17	***
Index of obesity	18.4±4.0	13.9±2.6	***
1975			
Height (cm)	160±6	162±6	n.s.
Weight (kg)	76.0±16.1	60.5±9.8	***
Relative body weight	1.25±0.28	0.97±0.17	***
Index of obesity	18.6±4.1	14.1±2.6	***

at the initial recordings in both groups ($p<0.01$). In 1975, systolic BP had decreased ($p<0.01$). Group A had returned to the initial level, while in group B the level remained higher ($p<0.01$). Diastolic BP varied less between the different occasions. It was somewhat lower in group A in 1975 than in 1969–70 ($p<0.05$) and higher in group B in 1972 than in 1969–70 ($p<0.01$) (Table II).

Biochemical variables

The B-hematocrit, fS-triglyceride, S-T₄ and S-urate levels were significantly higher in group A than in group B. There was no difference between the two groups in fB-glucose, fS-cholesterol or S-T₃ levels. The results in 1975 are given in Table III. The differences between the groups were similar in 1972 and 1975, although the absolute levels were not identical.

Intake of energy, fat, protein, carbohydrates, minerals, and electrolytes

The intake of energy-yielding nutrients—fat, protein and carbohydrates—is given in Table IV both

Table II. Blood pressures (mmHg) at the first study and at the follow-up examinations

	Group A	Group B
Systolic BP		
1969–70	175±34	114±8
1972	190±34**	130±15***
1975	179±32	123±19**
Diastolic BP		
1969–70	102±13	73±6
1972	103±14	76±7**
1975	97±13*	73±7

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table III. Biochemical measurements in 1975 for the two groups

	Group A	Group B	<i>p</i>
fS-Cholesterol (mmol/l)	5.9±1.1	5.5±1.0	n.s.
fB-Glucose (mmol/l)	4.8±0.4	4.6±0.5	n.s.
B-Hematocrit (%)	39±2	37±3	***
fS-Triglycerides (mmol/l)	1.10±0.67	0.77±0.59	*
S-Urate (μmol/l)	257±51	228±56	*
S-T ₃ (ng/ml)	1.79±0.27	1.72±0.20	n.s.
S-T ₄ (ng/ml)	93±19	84±12	*

in absolute amounts and in per cent of total energy. The mean values of energy and energy-yielding nutrients in groups A and B did not differ between the six sampling days and no significant differences between the groups were found for any of the studied sources of energy (Table IV). Both groups showed an average daily intake of energy around 6.3–6.4 MJ. In group A, 35% of the energy originated from fat and 38% in group B. Protein constituted 13–14% of the energy in both groups and carbohydrates around 50%.

Neither did the intakes of minerals (calcium, magnesium, zinc, copper) and electrolytes (sodium, potassium) differ between the groups (Table IV). The sodium intake in both groups amounted to around 100 mmol daily, corresponding to an intake of salt of about 6 g/day.

Urinary excretion of minerals, electrolytes and nitrogen

The mean values for urinary excretion of minerals (calcium, magnesium, zinc, copper), electrolytes (sodium, potassium) and nitrogen (protein) showed

Table IV. Energy intake during six sampling days

	Group A	Group B
Energy intake		
MJ	6.4±1.5	6.3±1.5
kcal	1525±349	1512±353
Fat (g)	58±16	62±18
	(35%)	(38%)
Protein (g)	52±15	48±11
	(14%)	(13%)
Carbohydrates (g)	189±42	180±45
	(51%)	(49%)
Sodium (mmol)	105±31	94±25
Potassium (mmol)	55±14	52±12
Calcium (mg)	577±287	577±224
Magnesium (mg)	270±85	243±66
Zinc (mg)	8.1±2.7	8.1±2.9
Copper (mg)	1.50±0.68	1.45±0.49

Table V. *Urinary excretion of minerals and protein (nitrogen $\times$ 6.25) in studies performed in 1972 and on two occasions in 1975*

	Group A	Group B
1972		
Sodium (mmol)	133 $\pm$ 43	120 $\pm$ 38
Potassium (mmol)	67 $\pm$ 24	56 $\pm$ 18
Protein (g)		56 $\pm$ 17
1975:1		
Sodium (mmol)	146 $\pm$ 54	125 $\pm$ 54
Potassium (mmol)	60 $\pm$ 19	59 $\pm$ 19
1975:2		
Sodium (mmol)	120 $\pm$ 32	118 $\pm$ 28
Potassium (mmol)	59 $\pm$ 12	59 $\pm$ 14
Calcium (mg)	146 $\pm$ 56	130 $\pm$ 61
Magnesium (mg)	67 $\pm$ 21	59 $\pm$ 25
Zinc (mg)	0.35 $\pm$ 0.13	0.27 $\pm$ 0.11
Copper (mg)	0.05 $\pm$ 0.016	0.05 $\pm$ 0.013
Protein (g)	58 $\pm$ 13	55 $\pm$ 12

no differences between groups A and B (Table V). The values for sodium and potassium were virtually unchanged in both groups between 1972 and the two examinations in 1975 (Table V). Analysis of variance did not show any significant differences between the separate measurements of 24-hour urinary excretions of sodium and potassium in groups A and B, respectively. (Group A: *F* ranged between 1.41 and 2.40, *DF*=(4.154); group B: *F* ranged between 0.17 and 0.30, *DF*=(4.161).) The mean urinary excretion of protein in group B in 1975:2 did not differ significantly from the value in 1972 (Table V). The mean values of sodium, potassium and protein in the food were lower ($p<0.001$) than the corresponding values for urinary excretion. The mean values of urinary excretion of calcium, magnesium, zinc, and copper were lower ($p<0.001$) than the consumption.

Correlations between some of the investigated parameters

The index of obesity was correlated to mean arterial BP in group A ($p<0.001$) but not in group B (Table VI). The index of obesity showed negative correlations with the intake of fat, protein and carbohydrates in group A ($p<0.01$) while corresponding correlations in group B were not significant (Table VI). Dietary protein, sodium and potassium were correlated to the corresponding urinary excretion values in groups A and B ($p<0.001$ or $p<0.01$)

(Table VI). The intakes of sodium and potassium showed negative correlations with mean arterial BP in group A ($p<0.001$ and $p<0.01$, respectively) while the corresponding correlations in group B were not significant (Table VI).

DISCUSSION

Epidemiological investigations indicate that relative body weight or other expressions for obesity display a strong association with hypertension (6). In the present study the index of obesity correlated significantly ($p<0.001$) to mean arterial BP in group A (high BP) but not in group B (low BP). It must be underlined that group A was recruited from the females with the highest BP values in the population not on treatment for hypertension. Therefore they do not represent a group with established hypertension. But they may represent females in the borderline phase of primary hypertension, where studies of parameters associated with development of hypertension ought to be of greatest interest.

The index of obesity was higher ($p<0.001$) in group A than in group B and corresponded to a mean difference in body weight of 16 kg between the groups. The higher index of obesity in group A could not be explained by any differences in the energy intake or the intake of separate energy-yielding components like fat, protein or carbohydrate. In fact, there were significant negative correlations ($p<0.01$) between the index of obesity and the in-

Table VI. *Correlation coefficients between some of the investigated parameters*

	Group A	Group B
Index of obesity/mean arterial BP	0.65***	0.12
Index of obesity/intake of fat	-0.49**	-0.06
Index of obesity/intake of protein	-0.48**	-0.04
Index of obesity/intake of carbohydrates	-0.49**	-0.28
Intake of protein/urinary excretion of protein	0.59***	0.52**
Intake of sodium/urinary excretion of sodium	0.74***	0.55**
Intake of potassium/urinary excretion of potassium	0.64***	0.64***
Intake of sodium/mean arterial BP	-0.58***	-0.07
Intake of potassium/mean arterial BP	-0.53**	-0.15

** $p<0.01$, *** $p<0.001$.

take of fat, protein and carbohydrate in group A but not in group B.

The difference in body weight between the groups may have to be traced far back in time, because the index of obesity was unchanged in both groups when examined in 1972 and 1975. The dietary habits were studied in 1975. The present finding that the obese and normal-weight women consumed similar amounts of energy is also in accordance with earlier reports (9). Keen et al. (16), in a recent study of nutrient intake in relation to adiposity and diabetes, also found a highly significant inverse correlation between food energy intake and adiposity in three normal British populations. It was suggested from their findings that higher degrees of adiposity are associated with food energy intakes lower than average and hence lower total energy expenditures. However, these cross-sectional data do not exclude the possibility of a relationship between development of overweight and a concomitant progression of BP towards higher values.

The etiology of obesity is however a field for speculation. One aspect discussed is the regulation of the metabolic rate by thyroid hormones. This must be remembered when a decrease in body weight is used as a tool in the treatment of high BP. The turnover and the consumption of energy may differ between those with a high and those with a lower index of obesity. It is of interest in this context that the S-T₄ level was significantly higher in group A than in group B (Table III).

Salt, another nutrient suggested to be associated with hypertension, was consumed in the same amount, about 6 g, in both groups. There was no correlation between salt intake and mean arterial BP, which is in accordance with previous findings of our group in elderly with a similar mean daily salt intake (4–6 g), using the duplicate portion sampling technique (4). The urinary excretion of salt was highly reproducible, as demonstrated by analysis of variance. The excretions of sodium and potassium were of the same magnitude in the two groups and correlated to the intake but exceeded this by about 1–2 g ($p < 0.001$).

Recently, Bing et al. (2) described 24-hour urinary excretion of sodium as a means of monitoring the salt intake in patients with essential hypertension. The relevance of this report for the present study lies in the value for urinary excretion, which was 156 mmol/24 hours. For age- and sex-matched controls the corresponding value was 152 mmol/24

hours. The finding that hypertensive patients and normotensive subjects do not differ in the 24-hour urinary excretion of sodium is also consistent with other studies (7, 14, 22, 32). However, conflicting results have been reported on the relationship between BP and urinary sodium excretion (1, 15, 35). Thus, Berglund et al. (1) found that up to a resting diastolic BP of 90 mmHg sodium excretion rose, in agreement with the theory of pressure diuresis (11). Above 90 mmHg, however, sodium excretion fell with increasing BP. It was suggested that the data indicated a curvilinear relationship between BP and salt intake. We actually found a very strong negative correlation between intake of sodium and BP in the group with high BP. This may be interpreted as an expression of such a curvilinear relationship even if the narrow BP distribution in the low BP group may have masked a correlation between BP and salt intake.

Before drawing further conclusions from this dietary investigation, the validity of the duplicate portion sampling technique has to be discussed. The validity may be evaluated by comparing the intake with the output of sodium, potassium and nitrogen. Their magnitudes ought to be the same if the subject is in metabolic balance and the intake and excretion have been monitored under satisfactory conditions, which are not yet clearly established. Sodium, potassium and nitrogen were excreted in amounts exceeding the intake, suggesting an underestimation of the food intake. There is also a technical explanation to consider. The same chemical methods of analysis were applied to very different raw materials, e.g. urine and mixed lyophilized and fat-extracted food homogenate powder. In fact, losses of about 10% of sodium and potassium have been found during treatment of the food materials (4). Another possibility is unsatisfactory conditions, e.g. duration, choice of days and number of days, etc., for comparison of intake and excretion.

All current methods for measuring dietary intakes have shortcomings in reflecting a true picture of the food intake, but when two groups are compared, these factors presumably affect both groups equally. Therefore it seems justifiable to conclude from the present data that dietary factors could not be implicated as a primary cause of discrepancies in BP or body weight between the two groups. This conclusion does not rule out the possibility that a diet therapy which reduces the body weight of an

obese patient with hypertension would not be successful. It must be mentioned that Parijs et al. (28) observed a slight decrease in BP when the intake of salt was halved in patients with hypertension.

Roughly speaking, both urinary and dietary measurements showed that the salt intake of these women with low and high BP, respectively, did not exceed 10 g/day. Salt restriction has been employed efficiently, as evidenced by the rice-fruit diet of Kempner, which contained approximately 200–500 mg of sodium per day (17). When salt restriction is used to reduce BP, the critical level for sodium intake seems to be very low—about 2–3 g/day as shown by hemodynamic investigations (10, 19). Therefore the dietary goals outlined for the US (20) with a recommended daily salt intake of about 8 g seem doubtful. The present data regarding salt intake do not support restriction of salt intake as a tool for decreasing BP.

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nutrient intake and health status of vegans.
Chemical analyses of diets using the
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Nutrient intake and health status of vegans.
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Nutrient intake and health status of vegans. Chemical analyses of diets using the duplicate portion sampling technique¹⁻³

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ABSTRACT A strict vegetarian diet [vegan diet (VD)] was investigated. Six middle-aged vegans (three men and three women) collected copies of 24-h diets using the duplicate portion sampling technique. By chemical analyses, the nutrient composition was determined in detail and compared with corresponding figures of a normal mixed Swedish diet. In the VD 30% of the energy originated from fat compared with 40% in normal Swedish mixed diet (MD). Linoleic acid was the dominant fatty acid (60% of total fat in VD versus 8% in MD). The VD contained 24 g protein/1000 kcal compared to 30 g/1000 kcal in MD, but the intake of essential amino acids by the vegans exceeded the recommendations. Dietary fiber was about 5 times higher in the vegan diet (29 versus 6 g/1000 kcal) and sucrose similar to MD (18 versus 21 g/1000 kcal). Among the inorganic nutrients the concentration of calcium (351 versus 391 mg/1000 kcal) and sodium (53 versus 49 mmol/1000 kcal) were similar in both types of diets but the amount of potassium (56 versus 30 mmol/1000 kcal), magnesium (300 versus 110 mg/1000 kcal), iron (9 versus 6.5 mg/1000 kcal), zinc (6.5 versus 4.7 mg/1000 kcal), and copper (2 versus 0.7 mg/1000 kcal) were nearly doubled. Iodine (39 versus 156 µg/1000 kcal and selenium (5 versus 17 µg/1000 kcal) were much lower in the VD, selenium even being undetectable in several 24-h diets. The VD was rich in folic acid (301 versus 90 µg/1000 kcal in MD) but the intake of vitamin B₁₂ was only 0.3 to 0.4 µg/day (MD: 3 to 4 µg/day). No clinical signs of nutritional deficiency were observed in the vegans. Serum protein levels of the vegans as well as their serum lipoproteins were near the lower range of the reference group. In addition, none of the vegans was overweight and their blood pressures were low for their age. *Am. J. Clin. Nutr.* 34: 2464-2477, 1981.

KEY WORDS Vegan diet, duplicate portion sampling technique, chemical analyses of nutrients, fatty acids, sterols, protein, amino acids, carbohydrates, dietary fiber, minerals, trace elements, vitamin B₁₂, folic acid, nutrient status, blood parameters

Introduction

Vegans eat no food of animal origin. With respect to global economy, vegans are of interest, as their dietary habit may offer clues to whether a strictly vegetarian diet is nutritionally adequate for maintaining health. In a few clinical studies (1-3) in Great Britain and the United States, the health status of vegans has been demonstrated not to differ from that of subjects consuming a mixed diet. In fact, detailed clinical studies (4) have raised the possibility that the vegan diet might have protective properties against certain disease. Attention has, for instance, been drawn to the hypocholesterolemic effect of vegetable

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protein (5). Patients with severe angina pectoris have been claimed benefit from vegan diets (6). Recently, Sanders (7) summarized the literature regarding the nutritive value of a vegan diet and the health status of vegans.

Information about the nutrient quality of a vegan diet is, however, usually based on calculations from food tables. In the present study, we have applied the duplicate portion technique, which is based on chemical analyses of duplicates of 24-h food collections as described previously (8, 9). Hence, the principal aim of the present investigation was to obtain more detailed data of the composition of dietary fats, sterols, proteins, carbohydrates, minerals, trace elements, and vitamins in the vegan diet. Trace elements in vegan diets have been investigated only with respect to iron (7). Although it is well known that the vegan diet is practically lacking in vitamin B₁₂, analysis of the actual daily intake by vegans has not been reported earlier (7).

A medical examination that included extensive clinical laboratory tests has been undertaken to evaluate the nutritional status of vegans in relation to the composition of their diet.

Materials and methods

Participants

Three healthy men and three healthy women (aged 49 to 55 yr) who had followed a vegan dietary regimen for at least 3 yr were studied. The six subjects were all selected and invited to Föllingegården by its director. Föllingegården is a vegan center in central Sweden where

a dietary regimen usually followed by Swedish vegans, has been established by its director (10).

Vegan diet

The vegan menu is very similar from day to day. The variation consist of a wide selection of fruits, berries, vegetables, roots, nuts, and pulses. All animal foods, including milk and dairy products, have been eliminated. Salt, sugar, pepper, coffee, and Indian tea are not used.

A typical menu of the vegan diet of Föllingegården is summarized in **Table 1**.

Food sampling

Two of us (IA and ID) visited Föllingegården to assess the degree of individual variation in food intake as a basis for the size of our food collection program. Based on our observations during this visit, we decided to include six subjects, each being studied during 4 consecutive days, and thus providing a total of twenty-four, 24-h samples for chemical analysis.

The six subjects were studied while staying at Föllingegården, and were therefore eating food from a common source. The participants were informed about the aim of the study and the duplicate portion sampling technique. The importance of maintaining the usual food habits was stressed. The volunteer was responsible for preparing a duplicate food sample over each 24-h period, duplicated as exactly as possible by visual measurement. In addition a protocol was made up every day in which the participant recorded the type of food consumed and rough estimations of the quantities consumed expressed as spoonfuls, cupfuls, etc.

The duplicate portion, including drinking fluids consumed between the meals, were collected and frozen as described earlier (8, 9).

Urinary samples

Urine samples were obtained on the same days as the food collections. The urine was collected in bottles containing 5 ml conc. HCl to prevent bacterial contamination and growth. The total daily volume was measured,

TABLE 1
A typical vegan diet at Föllingegården

Breakfast	Buckwheat porridge with sesame seed milk and powdered dates, stewed fruit (apple, apricot, plum, pear), stewed berries (strawberries, bilberries), dried fruit (dates, figs, raisins, apricots, plums), fresh fruit (apple, pear, plum), sprouts (wheat, sesame seed), lentils, flax seed, wheat bran.
Lunch	Raw food (tomatoes, cucumber, finely minced carrots, oak leaf, fermented mixed vegetables, white cabbage), mixed salads (boiled or raw common beets, boiled or raw potatoes, fermented cucumber, parsley, dill, garlic or fermented vegetables, tomatoes, and lettuce or white cabbage, fermented vegetables and leek), warm dishes often based on a stuffing mix.*
Dinner	Raw food and mixed salads and a hot soup boiled from roots and vegetables. The soup is served together with bread, such as buckwheat rolls, whole grain bread or crisp, hard rye bread. (Bread is only served at dinner).
Meals between	The bulk of the beverages is served between meals as water (body temperature), several kinds of herb teas, fruit, and vegetable juices

* Mixture of cereals, vegetable protein, soya flour, potatoe flakes, garlic powder, and spices (imported from West Germany).

TABLE 2
Clinical material

Subject	Sex	Age	Ht, cm	Wt, kg	Wt/H ²	Heart rate per min	Blood pressure mm Hg	Physical examination	Previous diseases or illnesses	Occupation
DV-1	F	54	160	62	15.1	72	120/80	Systolic murmur grade 1- 2 left J ₂₋₃	Eczema since age 2; joint pains as an adult; allergy to drugs.	Teacher, gymnastics
DV-2	F	53	169	54	11.2	80	110/70	Apical systolic murmur grade 3-4	Sepsis at age 1; lumbar disc prolapse; Angina pectoris; Rheumatic joint pains; gynecological operation (not cancer); upper respiratory infections; allergy.	Former nurse pensioner
DV-3	F	51	169	59	12.2	80	120/80	Left-sided divergent strabis- mus	Asthma; pyelonephritis; gynecological opera- tion (not cancer); upper respiratory infec- tions; gastritis	Waitress
DV-4	M	52	179	65	11.3	64	140/90	No pathology.	Appendicitis; syphilis at age 20; meningitis at age 20-39.	Engineer
DV-5	M	49	178	79	14.0	72	110/80	No pathology.	None.	Manager
DV-6	M	55	185	63	10.0	68	110/80	Emaciated. Numerous nevi over upper part of the body.	Lumbar disc prolapse; tonsillitis (tonsillec- tomy); accidents; gastrointestinal disorders; arthrosis; neck pain.	Papermill worker

and a representative sample of each 24-h urine collection was stored at -17°C until assayed.

Medical and laboratory examinations

At the time of the nutritional investigation, the six participants underwent a medical examination and completed a questionnaire regarding present and past illnesses and diseases. All examinations were performed by one of the authors (KB) and in **Table 2** the main findings are summarized.

Blood samples were taken for laboratory determinations. The analyses were performed using routine methods at the Department of Clinical Chemistry, University Hospital, Lund.

High-density lipoprotein (HDL) cholesterol was determined by enzymatic cholesterol measurements (11) in the supernatant obtained after precipitation of triglyceride-rich lipoproteins with dextran sulfate-MgCl₂ (12). Low-density lipoprotein (LDL) cholesterol was calculated according to the formula described by Friedwald et al. (13).

Analyses of food and urine solids

The analyses of nutrients and other food components were performed as described previously (8, 9). In summary, energy intake expressed as kcal was calculated from fat as $9.3 \times \text{g fat}$, from protein as $4.1 \times 6.25 \times \text{g nitrogen}$, and from carbohydrates as $4.1 \times \text{g fat-free powder minus protein and 7\% ash (carbohydrate by difference)}$. The data were rounded off to the nearest 50 kcal. Fat was measured after extraction with chloroform and evaporation. Fatty acid and sterol composition were analyzed on lipid extracts using gas chromatography. Protein was determined as nitrogen using a slightly modified Kjeldahl method. The amino acids were determined by GLC as trifluoroacetyl-butyl-esters after acid hydrolysis (14). *Tryptophan* was determined with a spectrofluorimetric method (15) and methionine and cysteine, by ion-exchange chromatography after performic acid oxidation before acid hydrolysis (16). Low molecular weight carbohydrates were assayed both with enzymatic methods and with GLC. Glucose was determined with a Tris-glucose oxidase reagent (17) and galactose with a galactose dehydrogenase-NAD⁺ system (18). Fructose was assayed as described by Southgate (19). Starch and sucrose were determined by measuring the formation of glucose after enzymatic hydrolysis with amyloglucosidase and β -fructosidase, respectively (Boehringer, Mannheim). Lactose was analyzed as galactose liberated after hydrolysis with β -galactosidase. GLC of trimethylsilyl derivatives (20) yielded values in very good agreement with the enzymatic methods (N-G. Asp, D. Birkhed, and C-G. Johansson, unpublished data). The GLC method also gives sorbitol. Dietary fiber was determined gravimetrically after sequential digestion with pepsin and pancreatin (21). Both water-soluble and water-insoluble components are included. The values given are corrected for the small residues of protein and starch remaining in the fiber fractions. Minerals and trace elements were analyzed after wetashing in concentrated acids. Sodium and potassium were measured with flame photometry. Iodine was analyzed by spectrophotometry (the Sandell-Kotloff reaction). Vitamin B₁₂ and folates were analyzed microbiologically using *Euglena*

gracilis and *Lactobacillus casei* (ATCC 7488), respectively, as test organism. Total folate was obtained after enzymatic hydrolyses. Nitrate was reduced to nitrite with cadmium and determined spectrophotometrically and calculated from total nitrite minus the nitrite concentration obtained before the cadmium reduction. Oxalic acid was determined by a potassium permanganate titration method.

Statistics

The differences between the groups were calculated with Student's *t* test.

Results and discussion

Nutrient intake

The nutrient intake by the six vegans is given both quantitatively and qualitatively in **Table 3**. As a qualitative measure, the nutrient densities (intake/1000 kcal) have been used. In these qualitative terms the normal mixed diet has been shown to be rather similar in different groups and independent of age and sex (9). The vegans were all middle-aged and a diet study of elderly was chosen as reference mainly because the differences between these two groups regarding age were probably of no significance. Recommended intakes of nutrients are for instance given in similar amounts to all over 51 yr.

Energy. The mean daily intake was 1700 kcal (7.1 MJ) and 1400 kcal (5.9 MJ) for male and female vegans, respectively (**Table 3**) when corrected for the dietary fiber included in the "carbohydrate by difference." Corresponding figures for the 68- to 70-yr-old men and women on normal mixed diets were respectively 2050 and 1600 kcal.

In their reviews Sanders (7) and Miller and Mumford (22), reported that vegans have a lower energy intake when compared to subjects on common diets in agreement with the present study. On the other hand, previous vegan studies have reported higher energy intakes compared with the present study. Differences in the food collection methods are probably the reason for such discrepancies. Considering the energy requirements of these age groups men and women should consume 2400 and 1800 kcal/day, respectively, according to Recommended Dietary Allowances (RDA) (23).

The energy-yielding components expressed in percentage of energy are shown in **Table 3**. In the vegan diet 29% originated from fat;

TABLE 3

Mean daily intake of energy and nutrients by three male and three female vegans each collecting duplicate portions during 4 consecutive days; the nutrient quality of the vegan diet expressed as nutrient density is given and also compared with ordinary mixed diet

Nutrients (Volunteers $\times$ diets)	Daily intake*		Nutrient density†		p‡ values
	Men (3 $\times$ 4)	Women (3 $\times$ 4)	Vegan diet (6 $\times$ 4)	Normal mixed diet (35 $\times$ 7)	
Energy (kcal)§	1,700 $\pm$ 250	1,400 $\pm$ 370			
Energy (MJ)§	7.1 $\pm$ 11	5.9 $\pm$ 1.6			
Total fat (g)	55 $\pm$ 12	56 $\pm$ 18	31 $\pm$ 6.7	44 $\pm$ 7	<0.001
(%)			29 $\pm$ 6	40 $\pm$ 5	<0.001
Linoleic acid (g)	31 $\pm$ 8	29 $\pm$ 12	17 $\pm$ 4.6	4.3 $\pm$ 2.3	<0.001
Total sterols (mg)	424 $\pm$ 81	338 $\pm$ 119	211 $\pm$ 52	184 $\pm$ 90	NS
Cholesterol (mg)	29 $\pm$ 12	27 $\pm$ 14	16 $\pm$ 7.2	140 $\pm$ 67	<0.001
Protein (g)	49 $\pm$ 8	37 $\pm$ 9	24 $\pm$ 2.4	30 $\pm$ 6	<0.001
(%)			10 $\pm$ 1	12 $\pm$ 2	<0.001
Total carbohydrate (g)	323 $\pm$ 49	231 $\pm$ 60	150 $\pm$ 14	105 $\pm$ 12	<0.001
(%)			60 $\pm$ 8	47 $\pm$ 5	<0.001
Sucrose (g)	43 $\pm$ 12	21 $\pm$ 7	18 $\pm$ 7	21 $\pm$ 10	NS
Dietary fiber (g)	62 $\pm$ 9	43 $\pm$ 9	29 $\pm$ 4	6.3 $\pm$ 2	<0.001
Sodium (mmol)	118 $\pm$ 23	75 $\pm$ 20	53 $\pm$ 12	49 $\pm$ 17	NS
Potassium (mmol)	124 $\pm$ 18	81 $\pm$ 16	56 $\pm$ 8.4	30 $\pm$ 6	<0.001
Calcium (mg)	725 $\pm$ 227	527 $\pm$ 206	351 $\pm$ 135	392 $\pm$ 94	NS
Magnesium (mg)	615 $\pm$ 90	468 $\pm$ 100	300 $\pm$ 29	112 $\pm$ 21	<0.001
Iron (mg)	17 $\pm$ 6	16 $\pm$ 7	9.0 $\pm$ 2.4	6.5 $\pm$ 1.9	<0.001
Zinc (mg)	13 $\pm$ 2.3	10 $\pm$ 3.5	6.5 $\pm$ 1.3	4.7 $\pm$ 1.5	<0.001
Copper (mg)	3.9 $\pm$ 0.8	3.3 $\pm$ 0.7	2.0 $\pm$ 0.34	0.7 $\pm$ 0.29	<0.001
Selenium (μ g)	6.9 $\pm$ 3.6	12.3 $\pm$ 22.5	5 $\pm$ 8	17 $\pm$ 12	<0.001
Iodine (μ g)	82 $\pm$ 29	58 $\pm$ 12	39 $\pm$ 13	156 $\pm$ 57	<0.001
Vitamin B ₁₂ (μ g)	0.39 $\pm$ 0.08	0.31 $\pm$ 0.09	0.19 $\pm$ 0.03	2.7 $\pm$ 2.0	<0.001
Total folate (μ g)	620 $\pm$ 120	470 $\pm$ 130	301 $\pm$ 46	90 $\pm$ 55	<0.001
Other food components					
Oxalate (g)	0.98 $\pm$ 0.22	0.84 $\pm$ 0.22	0.5 $\pm$ 0.18	0.16 $\pm$ 0.11	<0.001
Nitrate (mg as NaNO ₃)	169 $\pm$ 51	158 $\pm$ 38	92 $\pm$ 22	18 $\pm$ 8	<0.001
Nitrite (mg as NaNO ₃)	2.4 $\pm$ 3.2	3.3 $\pm$ 2.9	1.7 $\pm$ 1.3	1.7 $\pm$ 1.1	NS

* Mean $\pm$ SD.

† Amount per 1000 kcal, calculated on the energy values not corrected for dietary fiber.

‡ / test vegan diet versus normal mixed diet.

§ Corrected for dietary fiber.

| By difference, including dietary fiber.

10% from protein; and 61% from carbohydrates (by difference), with dietary fiber included. Corresponding values for the normal mixed diets were 40, 12.5, and 47.5%, respectively.

Previous studies on vegans (7, 22) have reported similar distributions of the energy-yielding substances, except that these studies showed a higher energy percentage from fat (35%) and a smaller one from carbohydrates (55%).

The mean food density (g/ml) was 0.13 for the women and 0.14 for the men, which should be compared with 0.20 obtained for normal mixed diets (9).

Fat. Both the male and female vegans consumed an average of 55 g fat/day. In the

elderly, corresponding intake was 90 and 71 g, respectively. The fatty acid composition differed markedly from ordinary mixed diets. As shown in **Figure 1**, linoleic acid predominated in the vegan diet, comprising to 50 to 60% of the total fatty acids, whereas oleic and palmitic acids were the dominant fatty acids in the normal mixed diet. The ratio between polyunsaturated and saturated fatty acids was 3.7 in vegan diet, but only 0.18 in the normal mixed diet. According to the written food protocols, safflower oil was used in salads and warm dishes.

Sterols. The daily intake of total sterols was on an average 424 and 338 mg for men and women, respectively, that was not significantly different from the amounts found in

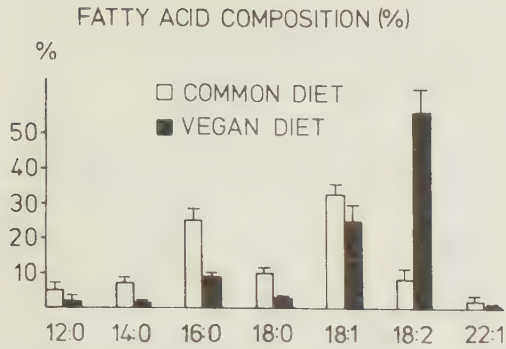


FIG. 1. Fatty acid composition in the vegan diet (■) compared with the ordinary mixed diet (□) expressed as percentages. Mean and SD.

the normal mixed diet when expressed per 1000 kcal. The sterol composition, however, was quite different (Fig. 2). In the vegan diet, sitosterol was the main sterol component making up approximately 75% of the total sterols, whereas cholesterol was less than 10%. In the normal mixed diets, the corresponding values were 17 and 75%, respectively. Expressed in nutrient densities, the vegan diets contained only about one-ninth as much cholesterol as the normal mixed diet when expressed per 1000 kcal.

Although minor amounts of cholesterol have been found in vegetable oils (24), it is possible that some of the material in the cholesterol fraction represents unidentified sterols, reported in several vegetable foods (25). Dietary plant sterols are poorly absorbed in the intestine (26) and lower plasma cholesterol when administered in large amounts. Thus the vegan diet contains little saturated fat and cholesterol and is rich in polyenoic fat and plant sterols. Such a diet fulfills the criteria of a plasma-lipid-lowering diet recommended by several committees (27). No rigorous scientific proof for the beneficial effects of a lipid-lowering diet is available at present.

Protein. The mean daily intake of protein was 49 and 37 g for the male and female vegans, respectively (Table 3). Expressed in g/1000 kcal, the vegan diet averaged 24 g, which is significantly lower than for normal mixed diet, amounting to 30 g/1000 kcal (Table 3). The lower protein intake by vegans compared to omnivores are in agreement with previous studies (7, 22).

The Swedish National Food Administration recommends a daily intake of 32 g protein/1000 kcal as a desirable level in planning diets, 28 g/1000 kcal as acceptable in evaluation of diets and 24 g/1000 kcal as a minimum level (28).

According to RDA (23), a daily intake of 0.8 g protein/day and per kg body weight is desirable. The mean consumption by the women in the present study was 0.54 (DV-1), 0.56 (DV-2), and 0.83 (DV-3) g/day per kg body weight. Corresponding figures for the three male vegans were 0.83, 0.54 and 0.77, for DV-4, DV-5 and DV-6, respectively. The 67-yr-old subjects of our previous study consumed 0.69 g/kg (men) and 0.68 g/kg (women).

In terms of egg and milk protein, the Joint FAO/WHO Expert Group consider 0.57 and 0.52 g/day/kg body weight, respectively (29), to be a safe level for adults, which for the reference man and women means a respective daily intake of 37 and 25 g protein only.

The protein intake in the present study is below international recommendations when its plant origin is taken into account. Moreover, the energy intake was low, which is known to influence protein requirements. Relating the protein intake to the urinary excretion of nitrogen (Table 4) the men, but not the women, were in positive nitrogen balance implying an adequate intake of protein as well as energy. Vegans, deliberately depress their protein intake because they believe that a high dietary protein intake has deleterious

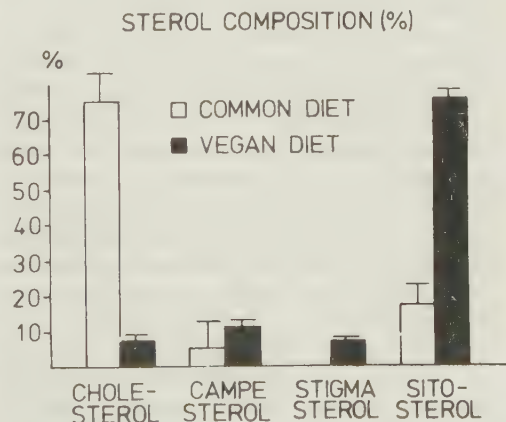


FIG. 2. Sterol composition in the vegan diet (■) compared with the normal mixed diet (□) expressed as percentages. Mean and SD.

TABLE 4

Comparison of intake and urinary excretion of nitrogen, sodium, and potassium; three male and three female vegans collected food and urine during 4 consecutive days

	Dietary intake*			Urinary excretion*		
	Men	Women	All	Men	Women	All
Nitrogen (g/day)	7.8 (1.3)	5.9 (1.6)†	6.8 (1.7)	6.8 (1.3)	7.6 (1.5)	7.2 (1.5)
Sodium (mmol/day)	118 (24)‡	75 (21)	96 (31)	82 (25)	88 (35)	85 (30)
Potassium (mmol/day)	124 (19)	81 (17)	103 (28)§	86 (28)	86 (26)	86 (26)

* Mean (SD).

† *t* test, intake versus excretion $0.001 < p < 0.01$.

‡ $p < 0.001$.

§ $p < 0.05$.

TABLE 5

The daily intake of essential amino acids, calculated as mg/kg body wt per day and for comparison corresponding data on ordinary mixed diets and the recommended intake of the essential amino acids for adults

	Vegan diet*	Ordinary mixed diet*	Vegan diet*	Ordinary mixed diet*	Recommendations†
	Men	Male pensioners	Women	Female pensioners	
Isoleucine	23 (17-31)	28 (19-52)	15 (14-24)	25 (13-38)	12
Leucine	29 (23-44)	55 (36-95)	26 (24-41)	48 (23-74)	16
Lysine	27 (21-28)	34 (19-57)	21 (18-31)	30 (14-76)	12
Total serum-containing	37 (22-40)	30 (23-41)	32 (32-38)	28 (24-33)	10
Total aromatic	35 (29-42)	42 (34-60)	24 (21-44)	40 (30-47)	16
Threonine	21 (18-24)	23 (11-39)	14 (14-23)	25 (12-38)	8
Tryptophan	11 (9-17)	8 (3-15)	10 (9-13.5)	8 (3-14)	3
Valine	26 (21-32)	32 (21-56)	19 (18-29)	29 (17-41)	14

* Median and range.

† RDA (23).

effects on the health. The health effects of the low protein intake are further discussed in relationship to clinical and biochemical results (see "Clinical and biochemical findings" below).

The intake of essential amino acids by the vegans was calculated as mg/day and kg body weight and compared with corresponding values for the elderly subjects on a normal mixed diet (Table 5). The intake of essential amino acids was found to exceed the recommendations set by the Food and Nutrition Board [RDA (23)]. It seems that food proteins of plant origin could supply all the essential amino acids when proteins from pulses are combined with protein from cereals and other vegetables (7).

Carbohydrate. The mean daily intake of total carbohydrate (by difference) was 323 and 231 g for the male and female vegans, respectively. The carbohydrate composition in vegan diets compared with normal diets is shown in Figure 3. Even though the vegans

do not use refined sugar, their intake of sucrose, expressed as g/1000 kcal was only 4 g lower when compared to normal mixed diets, which was a much smaller difference than was expected. There was, however, a striking difference between male and female vegans with regard to daily intake of sucrose, male vegans consuming twice as much as female vegans, 43 and 21 g sucrose per day, respectively.

According to food balance sheets, the sucrose consumption per capita in Sweden is 120 g/day, which corresponds to about 40 g/1000 kcal. In our studies on mixed diets, less than half of that amount was recorded (8, 9). The reason for this discrepancy is unclear but a few explanations have been tendered. The volunteers may have consumed less sugar during food sampling days than usual, the subjects participating in the study may be low sugar consumers and/or some of the sugar may be hydrolysed (inverted) during preparations of meals. The comparatively high

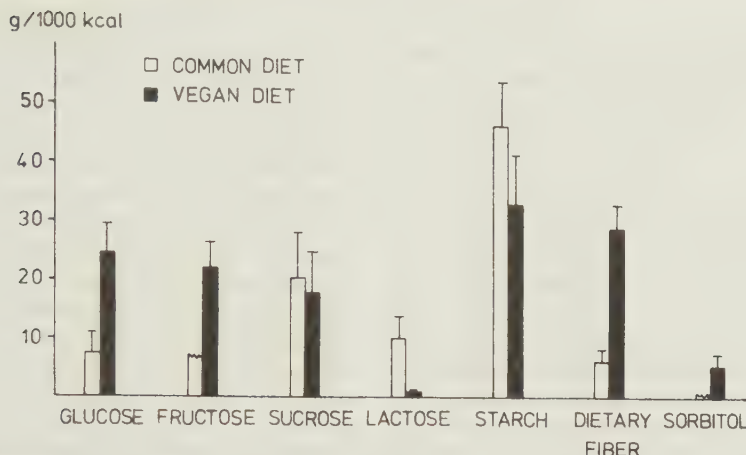


FIG. 3. Carbohydrate composition in the vegan diet (■) compared with the ordinary mixed diet (□) expressed in g/1000 kcal (nutrient density). Mean and SD.

amounts of sucrose in the vegan diet may be traced to fruits and berries consumed for breakfast as well as to vegetables, such as carrots, peas, etc.

The intake of monosaccharides, as with glucose and fructose, was 3 times higher than in mixed diets. This may also be due to fruits and might partially be a result of enzymatic or acid hydrolysis of sucrose or starch during preparation and storage of vegetables and fruits.

The starch content was somewhat lower than in the mixed diets, probably due to the fact that the vegans did not use bread from cereals.

Sorbitol constituted 5.4 ± 2 g/1000 kcal. Nine or 10 of the mixed diets earlier studied, contained only traces of sorbitol (<0.7 g/1000 kcal; D. Birkhed et al., unpublished observations). The rather high sorbitol concentration corresponding to a daily intake of about 10 g/day is still below the level at which gastrointestinal problems occur.

As expected, the vegan diet contained practically no lactose. The vegan diet is a high bulk diet. The concentration of dietary fiber/1000 kcal was about 5 times higher in the vegan diet (29 g/1000 kcal) as compared with normal mixed diets (6 g/1000 kcal). The mean daily intake of dietary fiber was 75 and 52 g for men and women, respectively. Harding and Stare (1) estimated that vegan diets contained on an average $2\frac{1}{2}$ times as much dietary fiber as normal mixed diets. Their

calculations were based on crude fiber values. During recent years more reliable techniques have been developed for analyzing dietary fiber (21), which also include the "water-soluble" part of dietary fiber.

Minerals and trace elements. Little information is currently available regarding both essential and toxic minerals and trace elements in vegan diet, except for iron (7). On the other hand, the levels in the diet per se do not provide information concerning the availability of these nutrients. Here, the medical and biochemical investigation of each subject in the present study may give valuable information (see below).

The mean daily intake of minerals and trace elements are shown in Table 3. Expressed in nutrient densities and compared with normal mixed diets, the vegan diet contained similar amounts of calcium and sodium 346 versus 394 mg/1000 kcal and 53 versus 49 mmol/1000 kcal, respectively. This was surprising because the vegans did not consume milk or dairy products and did not use any table salt.

The potassium content of the vegan diet was nearly doubled (56 versus 30 mmol/1000 kcal), and the magnesium was approximately 3 times higher (300 versus 112 mg/1000 kcal) when compared with normal mixed diets. In addition the vegan diet contained about 1.5 times higher concentrations of iron (9.0 versus 6.5 mg/1000 kcal), and zinc (6.5 versus 4.7 mg/1000 kcal), but 3 times higher concen-

trations of copper (2.0 versus 0.7 mg/1000 kcal). The higher amounts of certain minerals and trace elements may be because the vegan diet comprises of fewer refined food items, and that many vegetables and whole grain cereals are rich in minerals and trace elements.

A comparison of the daily intake of these nutrients with recommendations given by RDA (23) showed that the recommendation for magnesium (300 mg) was exceeded. Furthermore the male vegans consumed more than 10 mg iron daily, while the female vegans came very close to the recommended daily intake of 18 mg. Although the vegan diet was richer in zinc, the daily intake did not reach the recommended 15 mg for either male or female vegans. According to RDA (23), adults should consume 800 mg calcium daily. On the other hand, FAO/WHO (30) recommends a daily intake of 400 to 500 mg which was exceeded by the vegans.

The vegan diet was low in iodine, only 39 μg /1000 kcal versus 160 μg /kcal in normal mixed diets. Iodine occurs mainly in fortified table salt, marine fish, milk, and milk products, which were not consumed by the vegans. The iodine requirements have been estimated to be 1 μg /kg body weight per day (31). Five of the six vegans reached this level, but DV-1 did not. According to RDA (23), a daily intake of 90 μg iodine is recommended for women and 125 μg for men in these ages. These levels were not reached by the vegans in the present study, but no clinical signs of iodine deficiency were observed. Considering these low iodine intakes, one must keep in mind that the diet was collected at Föllingegården, which is situated in an area where the occurrence of endemic goiter was high before iodine fortification of table salt was introduced.

Also selenium was lower in the vegan diets averaging only 5 μg /1000 kcal compared with 18 μg /1000 kcal in normal mixed diets. On average, the female vegans consumed 6.9 μg selenium/day. Among the duplicate daily portions collected by the women, one contained undetectable levels, i.e., below 3 μg /daily portion. The highest concentration of selenium found was 13.3 μg /daily portion. Among the 12 daily duplicate portions collected by the male vegans, nine contained no detectable selenium. The other three dupli-

cate portions contained respectively 33, 47, and 66 μg .

It is known that food items of animal origin as for instance, fish, shellfish, and viscera are the richest sources of selenium, whereas cereals, vegetables, etc., are poor sources (32). Further, it has been demonstrated that Swedish soils, like those in all the Scandinavian countries, are low in selenium (32). No recommendation is presently available regarding the dietary needs of selenium in man, but the daily requirements have been estimated to be 100 to 150 μg (33). Normal mixed Swedish diets will supply a median intake around 25 μg /day (9), with a range from 10 to 95 μg . In a recent review of several studies on daily dietary intake of selenium in different countries ranges from 56 μg /day (New Zealand) to 326 μg /day (Venezuela) were estimated (34).

Vitamins. In the present study folate and vitamin B₁₂ were the only vitamins measured. The folate content averaged 3 times higher in the vegan diets than in the normal mixed diets, (301 versus 90 μg /1000 kcal), which was expected, since especially green vegetables are important sources of folate. RDA (23) recommends a daily intake of 400 μg which was exceeded by the vegans in the present study. According to FAO/WHO (35) a daily intake of at least 200 μg is sufficient.

The daily intake of vitamin B₁₂ was about 0.3 to 0.4 μg , which is approximately one-tenth of that in normal mixed diet. Vitamin B₁₂ is a product of microbiological synthesis and is not found in plant foods and should therefore not be present in vegan diets. Analyses of different vegan food items, including fermented vegetables, yielded vitamin B₁₂ values between 10 and 70 ng/100 g wet weight, which very well may explain the daily intake by the vegans. The small amount of vitamin B₁₂ recovered in the vegan diet may have been due to contamination with B₁₂-producing microorganisms present in food.

RDA (23) and FAO/WHO (35) recommends a daily intake of 3 and 2 μg of vitamin B₁₂, respectively, but according to Herbert (36) as little as 0.1 μg /day meets the minimum requirements. Vitamin B₁₂ deficiency is the most common nutrient deficiency described among vegans (37–41). Recently, the serious consequences for babies of vegan mothers have been described (42).

Nitrate-nitrite. The vegan diets contained 92 mg nitrate/1000 kcal versus 18 mg/1000 kcal in normal mixed diets. On the other hand, the nitrite levels were equal in both types of diets, being 1.5 to 2 mg/1000 kcal. The nitrate intake expressed per kg body weight per day varied between 1.6 and 3.4 mg and the nitrite intake between 0.03 and 0.10 mg/kg body weight per day. The acceptable daily intake of nitrate as NaNO_3 and nitrite as NaNO_2 is 5 and 0.2 mg/kg body weight per day, respectively (43). These acceptable daily intake levels were not exceeded by the vegans.

Oxalate. The vegan diet contained 3 times higher concentrations of oxalate compared with normal mixed diets. Almost 1 g/day was consumed by the vegans.

Validity of duplicate portion technique

To check the validity of the food collections using the duplicate portion sampling technique, the urinary excretion of sodium, potassium, and nitrogen was monitored and compared with corresponding intake values. The male vegans, but not the females, excreted significantly smaller amounts of sodium and potassium ($p < 0.001$) when compared to their intakes. Regarding nitrogen, there was no significant difference between intake and excretion by the male vegans. The women, however, excreted more nitrogen ($0.01 < p < 0.001$) than they consumed. The losses of nitrogen through the skin and in the faeces are not included in the excretion values tabulated in Table 4. These results indicate that the women but not the men underestimated their food collections. Correlation studies between intake and urinary excretion showed no significances for any of these nutrients.

Clinical and biochemical findings

Evaluation of the vegan diet may be made by comparisons with accepted nutrient requirements and recommendations. Another approach is to relate it to the clinical and biochemical status of vegans.

It has been reported that vegans have lower body weights than persons on normal mixed diets (3). None of the vegans in the present study was overweight, which is noteworthy in this age group (Table 2). The low intake of energy and fat in combination with a high

intake of dietary fiber might be the explanation for the absence of overweight but the role of physical exercise included in the vegan life style should not be dismissed.

The blood pressures of the vegans in the present study were low considering their ages (Tables 2). A high salt intake and overweight has been reported to be associated with high blood pressure. Obviously, these two risk factors are reduced by the vegan lifestyle.

Another consequence of the low fat diet high in bulk and polyunsaturated fatty acids may be the low concentrations of plasma lipids also reported by Sanders (7). As seen in Table 6, the concentrations of cholesterol, triglyceride, HDL, and LDL concentrations were in the lower half of the reference range. The mean ratios LDL-cholesterol/HDL-cholesterol were 1.8 (men) and 3.1 (women). The former values agree with findings in 30- to 40-yr-old vegetarians (44), whereas our ratio for the women was considerably higher. It should be emphasized that the reference ranges for the lipoprotein classes in Table 6 are not corrected for age; these 49- to 55-yr-old subjects could be expected to have LDL values in the upper reference range. A low LDL/HDL cholesterol ratio has been suggested to be advantageous from the atherogenic point of view (45) and may also reflect the preferential use of endogenous rather than exogenous fat.

Plasma prealbumin was low in DV-1 and DV-2 and borderline in DV-3, but was normal for the male vegans (Table 6). All six subjects had plasma albumin values near the borderline value of 38 g/l. This was probably due to a low protein intake or a low protein/energy ratio in the vegan diet. On the other hand, none of the subjects studied presented clinical or biochemical indications of protein deficiency. Their retinol-binding protein and ceruloplasmin for example were all normal (Table 6).

Two subjects had blood folate levels above normal (Table 7). Serum cobalamins were analyzed both with the radioimmuno assay technique and microbiologically using *E. gracilis* as test organism. One male vegan (DV-4), having been a vegan for the longest period of time, 10 yr, had a borderline value using the radioimmuno assay technique but was deficient in the microbiological test, while the other five subjects had serum cobalamins

TABLE 6
Laboratory examination

Parameter	DV 1	DV 2	DV 3	DV 4	DV 5	DV 6
Reference range						
Iron and vitamins						
Hb (g/l)	128	122	122	148	141	139
Serum iron (μ mol)	24	18	19	28	19	26
Serum, total iron binding capacity (μ mol/l)	43	54	44	42	40	38
Serum ferritin (g/l)	31	38	38	97	87	90
Blood folate (nmol/l)	153	176	221	153	184	>250
Serum cobalamin (pmol/l)	180*	190*	310*	110*	300*	280*
Plasma retinol-binding protein (mg/l)	118†	153†	255†	53†	194†	206†
	52	53	57	59	72	86
Lipids and lipoproteinstatus						
Serum triglycerides (fasting) (mmol/l)	0.8	0.6	0.7	0.6	0.8	0.9
Serum cholesterol (mmol/l)	4.4	5.3	3.5	4.4	4.7	5.8
HDL (mmol/l)	1.5	1.4	1.3	1.3	0.9	1.0
LDL (mmol/l)	2.6	2.9	2.0	2.6	3.1	3.7
LDL/HDL ratio	1.73	2.07	1.53	2.0	3.4	3.7
Plasmaproteins						
Plasma prealbumin (g/l)	0.09	0.17	0.18	0.24	0.21	0.26
Plasma albumin (g/l)	37	42	38	44	39	40
Plasma orosomucoid (g/l)	0.55-1.05	0.60	0.46	0.70	0.55	0.65
Plasma ceruloplasmin (g/l)	0.22-0.48	0.40	0.25	0.45	0.30	0.35
Plasma IgG (g/l)	7-15	11.0	9.0	15.0	12.5	12.5
Plasma IgA (immunoglobulin A, g/l)	0.5-3.0	1.7	1.3	2.0	1.7	0.9
Antichymotrypsin (%)	70-130	120	80	120	70	100
α_1 -Antitrypsine (g/l)	0.95-1.75	1.80	1.20	1.60	1.20	1.30
Liver						
Serum bilirubin (μ mol/l)	13	7	10	23	18	11
Serum glutamyl transferase (μ cat/l)	3-20	0.4	0.4	0.3	0.3	0.4
Serum alkaline phosphatase (μ cat/l)	<1.0	3.5	3.0	2.2	2.6	3.3
Serum aspartate aminotransferase (μ cat/l)	0.8-4.6	1.1	0.4	0.5	0.4	0.4
Electrolytes						
Serum sodium (mmol/l)	144	144	146	144	145	146
Serum potassium (mmol/l)	4.6	3.8	4.3	4.5	3.8	4.2
Serum magnesium (mmol/l)	1.0	0.9	0.9	0.9	1.0	1.0
Serum creatinine (μ mol/l)	65	65	80	80	90	80
Serum urate (μ mol/l)	290	325	320	355	340	335
Plasma creatinine clearance (ml/min)	93	65	64	71	73	74
Urate clearance (ml/min)	10.3	5.9	8.6	6.0	6.1	6.8
Reference range						
M: 131-163 W: 115-147						
10-30						
45-72						
>30						
70-200						
110-650						
40-80						
0.3-2.1						
3.7-7.2						
0.8-1.7						
1.3-4.5						
0.18-0.30						
38-52						
0.55-1.05						
0.22-0.48						
7-15						
0.5-3.0						
70-130						
0.95-1.75						
3-20						
<1.0						
0.8-4.6						
<0.67						
136-146						
3.5-5.0						
0.7-1.1						
60-115						
M: 180-480 W: 120-420						
75-160						

Parameter	Reference range	DV 1	DV 2	DV 3	DV 4	DV 5	DV 6
Other blood variables							
Serum triiodothyronin (nmol/l)	1.5-3.0	2.0	2.5	2.2	2.6	2.2	2.2
Serum thyroxin (nmol/l)	57-154	64	98	75	134	111	100
Serum thyroid stimulating hormone (working units)	<10	<1	4	6	2	<1	5
Serum T ₃ resin uptake (1 = 100%)	0.80-1.20	1.1	0.9	0.9	0.9	1.0	1.0
Blood glucose (fasting) (mmol/l)	3.3-5.6	5.0	4.4	4.2	5.5	4.3	4.4
Urine							
Volume/day (ml/day)		2820	1030	1880	875	1290	2360
Density (kg/l)		1.008	1.012	1.009	1.017	1.013	1.009
Calcium (mmol/day)	2.5-8	2.3	1.2	1.5	1.1	1.0	1.4
Phosphorus (mmol/day)		17	4	26	20	18	17
Creatinine (mmol/day)		8.9	6.0	7.3	8.2	9.4	8.5
Urate (μ mol/day)		4343	2740	3948	3063	2980	3304
Cortisol (nmol/day)	<900	550	160	360	180	230	190

Values below reference range are underscored.

* Radioimmuno assay technique.

+ *E. gracilis*.

within the normal range. Usually, man has vitamin B₁₂ stores to meet the daily requirements for about 3 yr and when small amounts of the vitamin is ingested daily it may take 5 to 10 yr or even longer before clinical signs of deficiency appears (7, 40).

The Hb-values were all within the normal range indicating sufficient absorption of the dietary iron. One had subnormal serum iron with normal transferrin and serum-ferritin values. Five of the six vegans had subnormal serum total iron-binding capacity.

Despite the low iodine intake, no clinical or laboratory parameter indicated any sign of iodine deficiency. S-TSH and T₃ values did not indicate hypothyroidism. The low intake of selenium among the vegans found in the present study is hard to evaluate. Specific symptoms of selenium deficiency in man is rarely reported. Recent studies from China indicate that dietary selenium deficiency may be associated with cardiomyopathy in children (46). Supplementation with 0.5 mg sodium-selenite/week in children 1 to 5 yr old and 1 mg/wk in children 6 to 9 yr old reduced the prevalence of the disease markedly. Selenium is an essential constituent of erythrocyte glutathione peroxidase in several animal species (33). It may play a role also in the plasma transport of tocopherol. At present, attention is being directed to the association of selenium deficiency with malignant diseases (47).

Bilirubin was increased in subject DV-4 and serum aspartate aminotransferase was increased in subject DV-2 without any other known hematological or hepatic disease.

Creatinine clearance was subnormal in five of six vegans. Serum creatinine values were normal, however. The urinary excretion of calcium and sodium (Tables 4 and 6) was lower than the normal range, whereas potassium was excreted in amounts above the normal range. This could be expected from their dietary habits.

Conclusion

The nutrient quality of the vegan diet differed in several respects from normal mixed diet. For some essential nutrients such as linoleic acid, the essential amino acids, calcium, iron, magnesium, potassium, copper,

and folic acid the recommendation set by either RDA (23) or FAO/WHO (29–31) were met, while for other nutrients like energy, total protein, zinc, iodine, and vitamin B₁₂ these recommendations were not met. In addition the selenium intake was very low, but the clinical significance is not clear at present.

Clinical and biochemical data of the vegans revealed no symptoms of nutritional deficiencies, except for one subject with a subnormal serum vitamin B₁₂.

Also other dietary components differed in vegan diet compared to normal mixed diet, for instance the vegan diet contained a high polyunsaturated/saturated fat ratio, a low concentration of saturated fat and cholesterol as well as high concentrations of dietary fiber and plant sterols, compared to normal mixed diet which probably contributed to certain clinical and biochemical findings in the vegans, e.g., low levels of blood lipids, low blood pressures, and normal to low body weights for their age. 

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*Nutrient intake and health
status of lactovegetarians.
Chemical analyses of diets
using the duplicate portion
sampling technique*



Nutrient intake and health status of lactovegetarians: chemical analyses of diets using the duplicate portion sampling technique¹⁻³

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ABSTRACT Six middle-aged lactovegetarians (three men and three women) collected copies of four 24-h diets using the duplicate portion sampling technique. By chemical analyses, the nutrient composition of the lactovegetarian diet was determined in detail and compared with that of a mixed Swedish diet (normal) and a vegan diet (vegan) studied previously. The nutrient composition of the lactovegetarian diet expressed per 1000 kcal represented an average between normal and vegan diets. It was in closer agreement with Swedish recommended dietary allowances than the normal Swedish diet. Thus, the lactovegetarian diet contained 35% of the energy as fat and was rich in polyunsaturated fatty acids, especially linoleic acid, which resulted in a polyunsaturated/saturated fat ratio of 0.6. The lactovegetarian diet had a cholesterol concentration only half of that of a normal diet. Protein content and amino acid composition were well above recommendations. The lactovegetarian diets contained less sucrose than normal and vegan diets, but the sum of the intake of low molecular weight carbohydrates was comparable to normal and vegan diets. Dietary fiber was three times higher than in a normal diet. Essential minerals and trace elements, ie, Ca, Mg, Na, K, Fe, Zn, Cu, Se, satisfied current requirements. The intake of vitamin B₁₂ by the lactovegetarians was around 1.4 µg daily, which is below the recommendations. The intake of folates was high, 300 to 400 µg daily. The clinical and biochemical investigation of the subjects revealed no signs of nutritional deficiency. Their plasma lipoproteins and the blood pressures were low for their age, in agreement with observations made earlier in a group of vegans. *Am J Clin Nutr* 1984;40:325-338.

KEY WORDS Lactovegetarian diet, duplicate portion, chemical analyses of nutrients, energy, fat, fatty acid, sterols, protein, amino acids, carbohydrates, dietary fiber, minerals, trace elements, vitamin B₁₂, folic acid, nitrate, nitrite, nutritional status, blood parameters

Introduction

During the last few decades, vegetarian diets have attracted considerable attention in discussions concerning human health and disease, and vegetarian diets have been proposed to cure or relieve symptoms of several chronic and disabling diseases (1-5). Vegetarian diets are subdivided in the vegan diet, ie, strictly vegetarian, accepting only foods of vegetable origin. The lactovegetarian diet

in addition includes milk products and lacto-ovo-vegetarian also accepts egg products.

Because of the suggested beneficial effects

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of vegetarian diets, it is of interest to know their nutrient composition as compared with an ordinary mixed diet. Such data are still scarce and most often incomplete. In a recent study, we investigated the nutrient composition of a diet collected in a vegan center in central Sweden, where a dietary regimen usually followed by Swedish vegans has been established. Using chemical analyses of duplicate portions of 24-h food collections, detailed information regarding the composition of fats, sterols, protein, carbohydrates, essential trace elements, and two vitamins (folic acid and vitamin B₁₂) were obtained (6). The nutrient composition of the vegan diet was compared with that of the ordinary mixed Swedish diet studied previously using the same food collection method (7).

The same food analysis method now has been applied to the lactovegetarian diet from a health center in central Sweden, where a dietary regimen usually followed by Swedish lactovegetarians has been established. The purpose of the present study was to compare the nutrient composition of the lactovegetarian diet with current dietary recommendations and also with the composition of the vegan diet (6) and ordinary mixed diet (7), both examined previously. In addition, the health status of the lactovegetarians as reflected by clinical and biochemical findings was studied.

Materials and methods

Participants

Six healthy volunteers, three men and three women (aged 46 to 58 yr), who had followed a lactovegetarian dietary regimen for 29 yr (median range 3 to 42 yr), were selected among the staff at a lactovegetarian health

center (Tallmogården, located in central Sweden). The protocol for this study was approved by the human subjects committee of the University of Lund.

Food sampling

The subjects collected foods during the same 4 consecutive days (September 1979). At each meal, everything consumed had to be duplicated as exactly as possible by visual measurements, and the duplicate was used for chemical analyses (6). The importance of maintaining the usual eating habits was stressed. In addition, a protocol was made up every day in which the participants recorded the type of food consumed and a rough estimation of quantities consumed expressed as spoonful, cupful, etc. A total of 24 duplicate portions, including drinking fluids consumed between the meals, were collected and frozen as described earlier (6, 7).

Urinary samples

Urine samples were obtained on the same day as the food collection. The urine was collected in bottles containing 5 ml conc HCl. The volume was measured, and a representative sample of each 24-h urine collection was stored at -17°C until assayed.

Medical and laboratory examinations

At the time of the nutritional investigation, the six participants underwent a medical examination and completed a standardized questionnaire regarding present and past illnesses and diseases. All examinations were performed by one of the authors (K-OA), and the main findings are summarized in Table 1. Blood samples were taken after 10 h of fasting for laboratory determinations. The analyses were performed at the Department of Clinical Chemistry, University Hospital, Lund, Sweden.

Analyses of food and urine solids

The analyses of nutrients and other food components were performed as described earlier (6, 7). Most components analyzed are shown in Table 2. Energy intake expressed as kcal was calculated from fat as $9.3 \times \text{g fat}$, from protein as $4.1 \times 6.25 \times \text{g nitrogen}$, and from carbohydrate as $4.1 \times \text{g fat-free powder minus protein and 7\% ash (carbohydrate by difference)}$. The data were rounded off to the nearest 50 kcal.

Results and discussion

Lactovegetarian diet

A typical lactovegetarian menu at the health center was as follows: breakfast usually included milk and porridge or different kinds of fermented milk, eg, yoghurt and buttermilk. In addition, several kinds of raw cereal, nuts, and fresh or dried fruits were consumed. Lunch was started with mixed salads of minced vegetables, roots, and pulses followed by a warm dish. Dinner also started with raw salads and was followed by

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TABLE 1
Clinical material

Subject	Sex	Age yr	Ht cm	Wt kg	Wt/ht ³	Heart rate per min	Blood pressure mm Hg	Physical examination	Previous disease or illness	Occupation
DLV-1	F	46	161	57	13.6	60	115/70	Normal findings	Only minor ailments	Registered nurse
DLV-2	M	52	168	63	13.3	70	120/70	Slight thoracic kyphosco- liosis	Tuberculosis before age 20. Not re- quiring hospitali- zation	Cook
DLV-3	F	58	168	64	13.5	70	130/80	Normal findings	Thyrototoxicosis at around age 30. Subtotal thyroid- ectomy	Cook, farmer
DLV-4	M	49	172	55	10.6	95	130/80	Normal findings	Pneumonia at 47 yr	Farmer using "biodynamic" techniques
DLV-5	F	46	160	63	15.4	80	135/85	Varicose veins in both legs below the knee	Varicose veins	Kitchen maid
DLV-6	M	49	179	71	13.0	54	105/60	Normal findings	Appendicitis; sub- clinical tuberculo- sis and jaundice before age 20; ur- ethritis and pros- tatitis around age 30	Physician

TABLE 2

Mean ($\pm$ SD) daily intake of energy and nutrients by three male and three female lactovegetarians each collecting duplicate portions during 4 consecutive days; the nutrient quality expressed as nutrient density (per 1000 kcal)

Nutrients	Daily intake		Nutrients density
	Men (3) 12 diets	Women (3) 12 diets	Men + women 24 diets
Energy (kcal)	2600 $\pm$ 530	1900 $\pm$ 380	
(MJ)	10.8 $\pm$ 2.2	8.0 $\pm$ 1.6	
Total fat (g)	98 $\pm$ 26	72 $\pm$ 26	37 $\pm$ 6.8
(%)	35 $\pm$ 6	35 $\pm$ 7	35 $\pm$ 6.5
Linoleic acid (g)	25 $\pm$ 9.2	13 $\pm$ 6.6	8.2 $\pm$ 3.4
Total sterols (mg)	436 $\pm$ 170	343 $\pm$ 115	174 $\pm$ 29
Cholesterol (mg)	151 $\pm$ 58	155 $\pm$ 60	69 $\pm$ 25
Protein (g)	64 $\pm$ 16	57 $\pm$ 12	28 $\pm$ 4.9
(%)	10 $\pm$ 2	12 $\pm$ 1	11 $\pm$ 2
Total carbohydrate (g)	346 $\pm$ 89	245 $\pm$ 44	132 $\pm$ 16
(%)	55 $\pm$ 6	53 $\pm$ 7	54 $\pm$ 6.6
Sucrose (g)	22 $\pm$ 14	14 $\pm$ 7	8 $\pm$ 5
Dietary fiber (g)	53 $\pm$ 17	33 $\pm$ 8	19 $\pm$ 5
Sodium (mmol)	104 $\pm$ 31	88 $\pm$ 45	43 $\pm$ 16
Potassium (mmol)	125 $\pm$ 32	87 $\pm$ 27	47 $\pm$ 9
Calcium (mg)	703 $\pm$ 236	1024 $\pm$ 265	429 $\pm$ 169
Magnesium (mg)	505 $\pm$ 156	350 $\pm$ 64	191 $\pm$ 37
Iron (mg)	16.0 $\pm$ 3.0	12.7 $\pm$ 4.0	6.6 $\pm$ 2.0
Zinc (mg)	11.5 $\pm$ 3.3	10.1 $\pm$ 1.4	5.0 $\pm$ 1.2
Copper (mg)	2.4 $\pm$ 0.8	1.8 $\pm$ 0.4	1.0 $\pm$ 0.2
Selenium (μ g)	68 $\pm$ 31	61 $\pm$ 17	30 $\pm$ 10
Vitamin B ₁₂ (μ g)	1.3 $\pm$ 0.5	1.5 $\pm$ 0.2	0.7 $\pm$ 0.2
Total folate (μ g)	442 $\pm$ 108	359 $\pm$ 122	182 $\pm$ 59
Other food components			
Nitrate (mg as NaNO ₃)	14.8 $\pm$ 6.6	11.2 $\pm$ 5.7	5.8 $\pm$ 2.9
Nitrite (mg as NaNO ₂)	11.6 $\pm$ 6.1	7.3 $\pm$ 2.2	4.2 $\pm$ 1.8

a hot soup. Bread was served with all meals. Most of the beverages, such as water, several kinds of herb teas, fruit and vegetable juices, were served between meals. All animal foods, except milk and dairy products were excluded. Salt, sugar, pepper, coffee, and Indian tea were not used.

In addition, four of the six subjects consumed a few nutrient supplements such as yeast (DLV-1), calcium (DLV-1, DLV-2, DLV-3), h-pantoten (DLV-2), wheat-germ (DLV-2), and an extract of fruits and vegetables containing minerals and vitamins (DLV-5). Some of these preparations, yeast, wheat germ, and calcium, were included in the collected food duplicates and may have contributed to some extent to their nutrient values. Since six staff members cannot be regarded as representative for lactovegetarians in general, it should be stressed that a main interest of the present study was to investigate the nutrient composition of a

lactovegetarian diet collected on a leading health center.

Validity of the duplicate portion sampling technique

To check the food sampling method, the intake and urinary excretion of protein (nitrogen), sodium, and potassium was compared. As seen in Table 3, the mean daily intake of nitrogen was 9.6 g, whereas the mean urinary excretion of nitrogen was 7.3 g, which was significantly lower ($p < 0.001$). If estimated nitrogen losses through the skin

TABLE 3
Daily nutrient intake compared with daily urinary excretion

	Daily intake*	Daily urinary excretion*	
Nitrogen (g)	9.6 $\pm$ 2.3	7.3 $\pm$ 1.3	$p < 0.001$
Sodium (mmol)	96 $\pm$ 39	97 $\pm$ 42	NS
Potassium (mmol)	106 $\pm$ 35	87 $\pm$ 35	NS

* Mean $\pm$ SD of 24 observations.

and feces, about 1.1 g daily (8, 9), were added to the urinary nitrogen, the excretion of nitrogen would be 8.4 g, which is still lower when compared with the intake. On the other hand, the intake and urinary excretion of sodium and potassium did not differ significantly (Table 3). Thus, it seems that the duplicate portions collected for chemical analysis agreed quantitatively with the portions actually consumed.

Energy

The mean energy intake by the men was 2600 and 1900 kcal by the women (Table 2). This intake is a 20 to 30% higher mean than that previously found in vegans (6) and pensioners adhering to a normal diet (7). Fat constituted, on an average, 35% of the dietary energy, protein 11%, and carbohydrate 54%. The proportion of fat energy was intermediate between that found in vegans (29%) and that for subjects on ordinary mixed diets (40%) (see Fig 1). Since the proportions of protein energy were similar, it follows that the lactovegetarians consumed more carbohydrates (54%) than subjects adhering to an ordinary mixed diet

(48%). In these calculations of energy intake, carbohydrates were calculated as carbohydrates "by difference." A still more valid estimate of energy intake is probably obtained if the calculated energy in dietary fiber (8% of total energy) is deducted, although some components in this group may contribute to available energy. The proportion of dietary fiber was approximately 3-fold higher than that found in the normal diet (6, 7).

Fat

Male and female subjects consumed, as a mean, 98 and 72 g fat/day, respectively (Table 2). This corresponded to 35% of the energy intake, with the individual means ranging between 29 and 41%. In a review of some previous studies (10), a fat intake among vegetarians ranging between 7 and 35% of dietary energy was found, the larger value being more typical. Also among Swedish female vegetarians, a lower fat intake was observed compared with controls (11).

Among fatty acids, octadecanoic acid (mainly oleic acid) was most prominent (34% of total fatty acids). The proportions

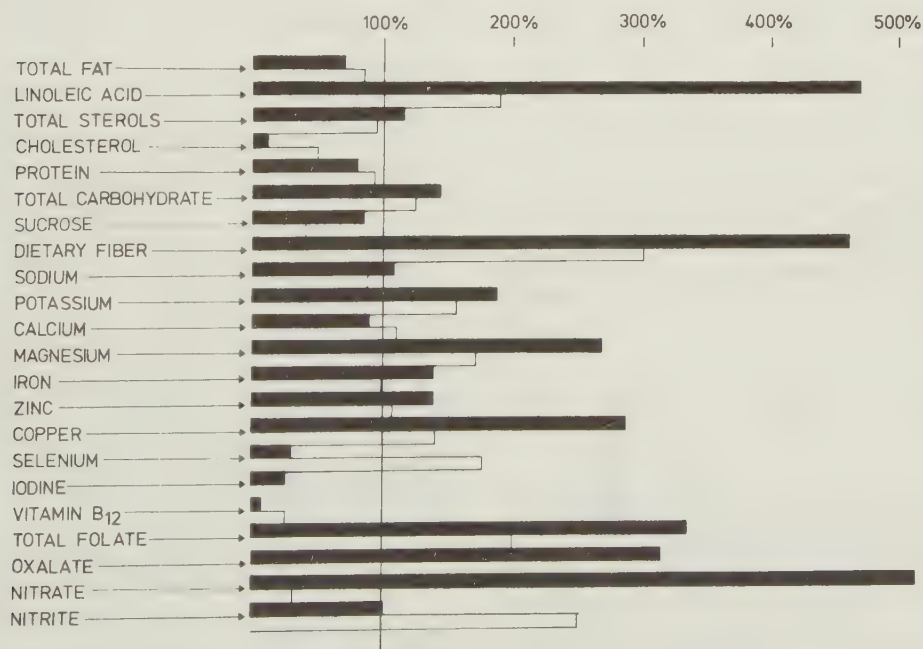


FIG 1. The nutrient quality of the vegan diet (■) and lactovegetarian diet (▨) expressed as percentages of the ordinary mixed diet (100%) calculated from the nutrient density data given in Table 2 of the present study and from two previous studies (6, 7).

of the four major long-chain saturated fatty acids (12:0 to 18:0) were generally similar to those found in the normal diet, although they together comprised only 39%, as compared with 47% in the pensioners' diet (Fig 2). Together with the several-fold higher intake of octadecadienoic acid (mainly linoleic acid) among lactovegetarians, this resulted in a higher polyunsaturated/saturated fat (P/S) ratio in the lactovegetarian diets (0.57), compared with normal diets (0.18), but lower than in vegan diets (3.7) (6). Several minor fatty acids, such as palmitoleic acid, occurred in relatively smaller quantities in the lactovegetarian diets. In a previous study, a P/S ratio of 0.36 to 0.45 was found for a lactovegetarian and 1.21 to 1.38 for a pure vegetarian diet (3). It is noteworthy that the dietary P/S ratio (0.57) found here is close to the most recent Swedish Nutritional Recommendation (P/S ratio approx 0.5; Reference 11). As reported previously, 3.9% of total dietary fatty acid intake was *trans*-octadecenoic acid, compared with 5.0% in the normal diet and 1.8% in the vegan diet (12). The intake of different positional fatty acid isomers has not yet been studied. Very little is known about the possible physiological effects of *trans*-isomeric fatty acids in humans.

Sterols

The total sterol intake was in the same range as that found for vegans and subjects on the normal diet (Table 2). The proportions of cholesterol and sitosterol, the major plant sterol, were approximately equal (Fig 3), and the intake of cholesterol was approx-

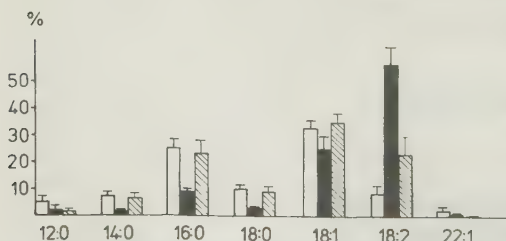


FIG 2. Fatty acid composition in the lactovegetarian diet (▨) compared with ordinary mixed diet (□) and vegan diet (■) expressed as percentages of total fatty acids. Mean and SD are shown. Data for ordinary mixed diet and vegan diet are taken from two previous studies (6, 7).

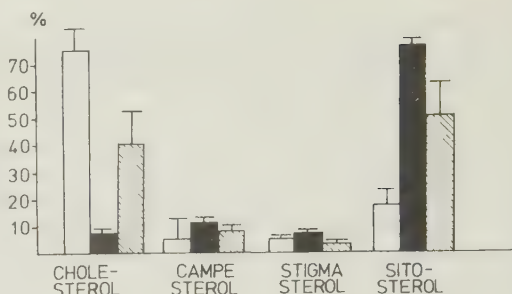


FIG 3. Sterol composition in the lactovegetarian diet (▨) compared with the ordinary mixed diet (□) and vegan diet (■) expressed as percentages of total sterol. Mean and SD are shown. Data for ordinary mixed diet and vegan diet are taken from two previous studies (6, 7).

imately half of that for the normal diet (69 versus 140 mg/1000 kcal). The intake of cholesterol by lactovegetarians can probably be explained by the content of cholesterol in the milk consumed (140 mg/kg) and in related foods.

Although phytosterols are poorly absorbed in the intestine, consumption of phytosterol-rich diets increase the amount of these sterols in serum. No physiological effects of such an increase have hitherto been demonstrated (13).

Protein and amino acids

Men and women consumed on an average 64 and 57 g/day, respectively, and the protein content per 1000 kcal was on an average 28 g (Table 2). The lactovegetarian diets contained more protein per 1000 kcal than the vegan diets, but slightly less than the ordinary mixed diets (Fig 1).

The average protein intake of lactovegetarian men and women, expressed as g/kg body weight and day, was 1.04 and 0.93, respectively. Corresponding data for the vegans were 0.77 and 0.54 g/kg and for pensioners on an ordinary mixed diets 0.69 and 0.67 g/kg (6, 7). Comparing with the recommended dietary allowances (14) of 0.57 g/kg for adult males and 0.52 g/kg for adult females, it is obvious that the lactovegetarians consumed more than an adequate amount of protein.

In Table 4 the composition of the essential amino acids per g protein is shown together with the reference protein according to

TABLE 4

Essential amino acid content of 1-day mixed-food diets expressed per mg/g protein*

Amino acid	FAO Reference protein	Ordinary, mixed diets† (n = 84)	Lactovegetarian diets (n = 24)	Vegan diets† (n = 24)
Isoleucine	40	36 ± 5.8	34 ± 11.3	31 ± 10.3
Leucine	70	70 ± 10.3	76 ± 21.4	50 ± 14.7
Lysine	55	43 ± 8.9	55 ± 15.8	36 ± 8.7
Phenylalanine + tyrosine	60	59 ± 15.0	108 ± 30.6	50 ± 7.4
Methionine + cysteine	35	41 ± 5.7	26 ± 7.8	49 ± 24.7
Threonine	40	32 ± 6.3	44 ± 11.0	28 ± 8.7
Valine	50	41 ± 4.9	47 ± 14.2	36 ± 10.5
Tryptophan	10	10 ± 5.1	9 ± 2.8	7 ± 5.7
Chemical score		78	74	65

* Mean ± 1 SD.

† Data from ordinary mixed diet and vegan diet are taken from previous studies (6, 7).

TABLE 5

Essential amino acid intake expressed in mg/kg body weight/day (mean ± 1 SD)

Amino acid	RDA	Ordinary, mixed diets* (n = 84)		Lactovegetarian diets (n = 24)		Vegan diets* (n = 24)	
		Male (6)	Female (6)	Male (3)	Female (3)	Male (3)	Female (3)
Isoleucine	12	28 ± 5.7	25 ± 4.8	33 ± 10.2	33 ± 12.1	23 ± 8.0	15 ± 6.3
Leucine	16	55 ± 10.1	48 ± 8.9	78 ± 21.3	80 ± 23.0	29 ± 13.3	26 ± 5.7
Lysine	12	34 ± 8.3	30 ± 8.7	57 ± 16.0	58 ± 15.7	27 ± 18.6	21 ± 7.3
Phenylalanine + tyrosine	16	42 ± 14.4	40 ± 15.0	112 ± 28.3	117 ± 31.0	35 ± 15.6	24 ± 11.7
Methionine + cysteine	10	30 ± 5.9	28 ± 4.7	27 ± 7.2	25 ± 6.9	37 ± 15.0	32 ± 14.8
Threonine	8	23 ± 8.9	25 ± 3.6	46 ± 10.9	58 ± 14.8	21 ± 12.0	14 ± 7.3
Valine	14	32 ± 4.9	29 ± 5.3	48 ± 14.0	38 ± 13.8	26 ± 13.2	19 ± 7.8
Tryptophan	3	8 ± 3.4	8 ± 4.1	9 ± 3.1	8 ± 2.9	11 ± 4.3	10 ± 5.4

* Data from ordinary mixed diet and vegan diet are taken from previous studies (6, 7).

FAO/WHO (15). The lactovegetarian diet was not low in lysine, but the amount of the sulfur-containing amino acids limited the protein quality. The limiting amino acid in the vegan diets and in the ordinary mixed diets was lysine.

The average intake of essential amino acids in mg/kg body weight and day exceeded the recommended intakes for both men and women regardless of type of diet (Table 5). Thus, the diet of the lactovegetarians in the present study contained an acceptable amount of protein of good quality. Biochemical findings in Table 6 regarding serum albumin and prealbumin along with other plasma proteins give further support of an adequate intake of dietary protein by these lactovegetarians.

Carbohydrates

The mean daily intake of carbohydrates (by difference) was 346 g for male and 245 g for female lactovegetarians (Table 2). Ex-

pressed as g/1000 kcal, this diet had a higher carbohydrate content than a normal Swedish mixed diet but lower than a pure vegan diet (Fig 1).

In Table 7 the composition of the dietary carbohydrates is presented. The sum of carbohydrates analyzed was more than 10% lower than carbohydrates calculated by difference. A part of this discrepancy could be explained by the fact that oligosaccharides, such as raffinose, stachyose, and verbascose, were not assayed. This class of carbohydrates is present in beans and lentils, for example, which constitute a considerable part of the lactovegetarian diet.

Starch constituted almost half of the total carbohydrates, and was thus the type of carbohydrate present in the highest amount. The mean intake was 105 g/day (as glucose; this value also includes maltose and dextrins). A major part of the starch was probably derived from cereals, since lactovegetarians include bread in all of their meals

TABLE 6
Laboratory examination

Parameter	Reference range	DLV-1 F	DLV-2 M	DLV-3 F	DLV-4 M	DLV-5 F	DLV-6 M
Iron and vitamins							
Hb (g/l)	M: 131-163 F: 115-147	155	140	155	155	130	165
Serum iron ($\mu\text{mol/l}$)	10-30	25.6	20.1	29.9	19.9	25.3	40.6*
Serum total iron-binding capacity ($\mu\text{mol/l}$)	45-72	56	59	70	53	71	67
Blood folate (nmol/l)	70-200	175	330	285	375	40	620
Serum cobalamin (pmol/l) (RIA) (<i>Euglena gracilis</i>)	110-650	320	180	250	370	255	190
Plasma retinol binding protein (mg/l)	40-80	233 41	147 52	200 60	312 43	207 33	142 57
Lipids and lipoproteins							
Serum triglycerides (mmol/l)	0.3-2.1	0.8	1.2	0.7	0.8	1.0	1.6
Serum cholesterol (mmol/l)	3.2-7.2	5.2	5.5	7.0	5.1	5.3	4.7
HDL (high density lipoproteins) (mmol/l)	0.8-1.7	1.3	0.7	1.3	1.2	1.1	1.0
LDL (low density lipoproteins) (mmol/l)	1.3-4.5	3.2	4.1	4.9	3.4	3.8	3.1
LDL/HDL ratio		2.5	5.9	3.8	2.8	3.5	3.1
Electrolytes							
Serum sodium (mmol/l)	136-146	145	148	150	145	145	145
Serum potassium (mmol/l)	3.5-5.0	4.1	4.5	3.9	5.0	4.1	3.6
Serum magnesium (mmol/l)	0.7-1.1	1.0	1.0	0.9	1.0	0.9	1.0
Serum creatinine ($\mu\text{mol/l}$)	60-115	84	106	110	102	60	81
Serum urate ($\mu\text{mol/l}$)	M: 180-480 F: 120-420	244	336	398	365	330	337
Plasma creatinine clearance (ml/min)	75-160	69	65	55	61	71	94
Urate clearance (ml/min)		3.8	2.8	1.6	1.9	3.4	2.5
Plasma copper (mg/l)		0.80	0.80	0.90	1.25	0.90	0.75
Plasma zinc (mg/l)		0.75	0.80	0.80	1.00	0.60	0.80
Blood zinc (mg/l)		4.00	5.50	7.25	6.50	5.25	5.00
Cu/Zn ratio		1.07	1.00	1.13	1.25	1.50	0.94

Other blood variables

Serum triiodothyronine (nmol/l)	0.9-3.2	1.9	2.1	2.1	2.3	2.3	2.4
Serum thyroxine (nmol/l)	57-154	124	92	128	116	80	125
Serum thyroid stimulating hormone (working units)	<10	2.0	2.0	3.0	3.0	3.0	2.0
Serum T ₃ resin uptake (1 = 100%)	0.80-1.20	0.98	1.06	0.91	0.96	0.89	1.14

Plasma proteins

Plasma prealbumin (g/l)	0.18-0.30	0.17	0.18	0.21	0.15	0.15	0.29
Plasma albumin (g/l)	37-52	38	43	42	39	39	45
Plasma orosomucoid (g/l)	0.55-1.05	0.60	0.70	0.90	1.20	0.75	0.60
Plasma ceruloplasmin (g/l)	0.22-0.48	0.30	0.30	0.35	0.45	0.35	0.30
Plasma immunoglobulin G (g/l)	7-15	14.0	16.0	13.0	12.0	16.0	12.0
Plasma immunoglobulin A (g/l)	0.5-3.0	1.6	1.1	1.1	2.1	1.2	1.6
α_1 -antitrypsin (g/l)	0.90-1.70	1.30	1.40	1.60	1.70	1.40	1.10

Liver

Serum bilirubin (μ mol/l)	3-20	15	11	12	11	9	40
Serum glutamyl transferase (μ cat/l)	<1.0	0.2	0.2	0.1	0.2	0.1	0.3
Serum alkaline phosphatase (μ cat/l)	0.8-4.6	2.0	3.6	3.1	3.2	4.2	3.4
Serum aspartate aminotransferase (μ cat/l)	<0.67	0.2	0.4	0.5	0.3	0.5	0.4

Urine: (mean of 4 observations)

Volume/day (ml/day)	1660 $\pm$ 222	760 $\pm$ 133	1300 $\pm$ 271	1420 $\pm$ 150	1320 $\pm$ 209	2320 $\pm$ 1086
Density (kg/l)	1.014 $\pm$ 0.003	1.023 $\pm$ 0.003	1.014 $\pm$ 0.002	1.016 $\pm$ 0.001	1.014 $\pm$ 0.004	1.013 $\pm$ 0.010
Calcium (mmol/day)	3.5 $\pm$ 0.3	5.9 $\pm$ 2.5	1.7 $\pm$ 0.3	2.2 $\pm$ 0.6	0.6 $\pm$ 0.2	2.8 $\pm$ 0.5
Phosphorus (mmol/day)	22 $\pm$ 4	24 $\pm$ 5	23 $\pm$ 1	25 $\pm$ 3	22 $\pm$ 2	23 $\pm$ 3
Creatinine (mmol/day)	8.3 $\pm$ 0.3	10.1 $\pm$ 0.3	8.6 $\pm$ 0.1	9.0 $\pm$ 0.7	5.9 $\pm$ 0.4	11.3 $\pm$ 2.0
Urate (μ mol/day)	1120 $\pm$ 214	1030 $\pm$ 330	1010 $\pm$ 79	1030 $\pm$ 106	1270 $\pm$ 446	1060 $\pm$ 387
Cortisol	460 $\pm$ 91	340 $\pm$ 57	360 $\pm$ 180	500 $\pm$ 48	270 $\pm$ 43	590 $\pm$ 170

* Data outside the reference range are underscoring.

TABLE 7
Intake of digestible carbohydrates and dietary fiber in duplicate portions of a lactovegetarian diet*

Carbohydrate	Intake	Intake
	g/1000 kcal	g/day
Glucose	15.2 $\pm$ 6.2	36.0 $\pm$ 20.3
Fructose	17.2 $\pm$ 5.3	39.9 $\pm$ 18.6
Galactose	0.3 $\pm$ 0.1	0.7 $\pm$ 0.2
Sucrose	8.1 $\pm$ 4.9	18.4 $\pm$ 11.9
Maltose	3.8 $\pm$ 2.4	8.4 $\pm$ 5.3
Lactose	7.6 $\pm$ 3.6	16.1 $\pm$ 5.9
Sorbitol	1.3 $\pm$ 1.1	2.9 $\pm$ 2.5
Starch	46.7 $\pm$ 8.9	105.4 $\pm$ 26.1
Dietary fiber	18.6 $\pm$ 4.7	43.4 $\pm$ 16.4

* No of samples = 24, mean $\pm$ 1 SD.

and also had cereal flakes or porridge for breakfast. The starch content was higher than in the mixed and vegan diets. Since products with a high starch content usually also are good sources of essential nutrients and fiber, it is often recommended that as large a part as possible of the dietary carbohydrates be present as starch.

Although the starch intake was high, the sum of the intake of low molecular weight carbohydrates was still higher. Glucose and fructose were the most abundant sugars, the mean intakes being 36 and 40 g/day, respectively. Other sugars present in high amounts were lactose (16 g/day), maltose (8 g/day), and sucrose (18 g/day). Compared with the diets studied earlier, glucose intakes were higher than in mixed diets. The intake of

sucrose was lower than in any of the diets studied earlier (Fig 4).

The lactose content in the lactovegetarian diet was similar to that in a mixed diet. Women had a higher lactose intake than men, 19 and 14 g/day, respectively. Nineteen grams lactose corresponds to 390 ml milk, and this amount of milk provided 24% of the protein content and 45% of the calcium content in the lactovegetarian diet. In the males' diet, milk provided 16% of the protein and 47% of the calcium. Thus, milk is a very important part of the lactovegetarian diet. In addition, lactose-free dairy products such as cheese were also consumed.

The mean intake of dietary fiber was 43 g/day. Per energy unit, this is three times that in a mixed diet but only two-thirds of that in a pure vegan diet (Fig 4).

Lack of dietary fiber has been put forward as one of the factors responsible for several diseases in affluent societies, such as constipation, diverticulosis, IHD, gallstones, colonic cancer, etc. Besides, dietary fiber flattens the curves of postprandial blood glucose and insulin both in test meals and in glucose tolerance tests (16).

On the whole, the composition of the carbohydrates in this lactovegetarian diet is well balanced from a nutritional point of view, with high intakes of starch and dietary fiber and a low intake of sucrose.

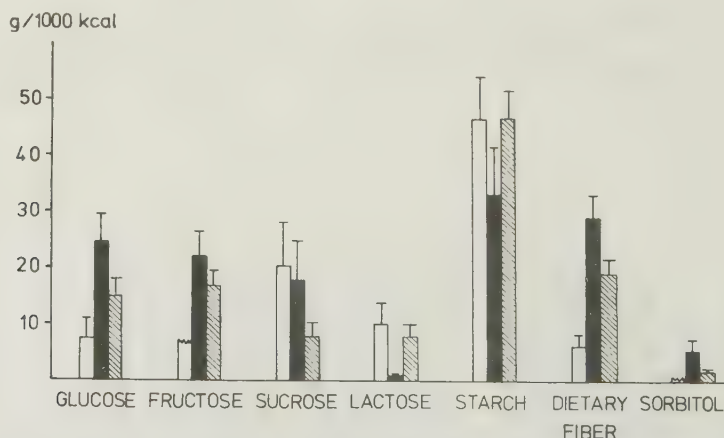


FIG 4. Carbohydrate composition in the lactovegetarian diet (▨) compared with the normal mixed diet (□) and vegan diet (■) expressed as percentages. Mean and SD are shown. The fructose content of the mixed diet was estimated to be in the same range as the glucose content. Data for ordinary mixed diet and vegan diet are taken from two previous studies (6, 7).

Minerals and trace elements

The mean intake and the nutrient density of essential minerals and trace elements are shown in Table 2 and Figure 1. The mean intake of calcium was 703 and 1024 mg/day for the men and women, respectively, which was somewhat higher than that found in the vegan and normal diets (Fig 1), and was probably due to the relatively high consumption of milk and milk products. The higher calcium intake by the women was due to a higher intake of dairy products, as also reflected by their higher lactose intake.

The mean daily intake of sodium by the men was 104 mmol and by the women 88 mmol, which was equivalent to 5 to 7 g of table salt. Because the lactovegetarians, like the vegans, do not add table salt to their meals, we expected a subnormal intake, but the intake was even slightly higher than found in the other groups (Fig 1). The potassium content found in the present study were higher than in ordinary mixed diets, but lower than in vegan diets (Fig 1). The same tendency was observed regarding magnesium (Fig 1). It appears that the lactovegetarians did not consume as much leafy vegetables and cereals as the vegans. The intake of minerals and electrolytes exceeded the recommended intakes (14).

The male lactovegetarians had a mean intake of iron of 16 mg/day, which was about the same as in male vegans (17 mg/day). The vegan females had a mean iron intake of 16 mg/day, which was very close to the RDA levels (18 mg/day), whereas the female lactovegetarians had a mean intake of 13 mg/day (Table 2). The blood Hb levels in the women were, however, within normal range (Table 6). The mean intake of zinc was 11.5 and 10 mg/day for men and women, respectively, which was close to that in the vegans, but slightly higher than found in the normal diet (Fig 1). The same tendency was observed in the case of copper. The vegan diet contained copper concentrations that were three times higher when compared with the normal diet, but the lactovegetarian diet contained only 40 to 50% more (Fig 1).

One of the most striking observations found in the present study concerns the intake of selenium. The mean selenium intake

was 68 and 61 $\mu\text{g/day}$ for the men and women, respectively, which was almost twice the levels observed in ordinary mixed diets. Previously, we reported that the intake of selenium by vegans and pensioners was far below RDA levels (6, 7). Some vegan diets had selenium levels less than 10 $\mu\text{g/day}$. This great difference might partly be explained by the fact that many of the food items served in the health center were imported, and it is possible that these products were grown in areas that are rich in selenium. The Food and Nutrition Board (14) has recommended a daily intake for adults of between 50 and 200 μg selenium, and it appears that the intake levels observed in the present study are adequate.

To summarize, the lactovegetarian diet provided most of the inorganic elements in amounts close to or above recommended intakes according to RDA (14). Plasma levels of some of the inorganic elements were within the normal range indicating a satisfactory intake and bioavailability of these elements (Table 6), although the plasma levels alone may not necessarily indicate the body status.

Vitamins

Among the vitamins, vitamin B₁₂ and folic acid were analyzed. Because vitamin B₁₂ is synthesized only by microorganisms, it occurs in animal foods almost exclusively. The trace amounts in vegetable foods probably originate from contaminating vitamin B₁₂-producing microorganisms (17).

The three male lactovegetarians consumed 1.3 $\mu\text{g/day}$ of vitamin B₁₂, whereas the corresponding figure for the women was 1.5 $\mu\text{g/day}$. The lactovegetarian diets contained 0.7 μg of vitamin B₁₂ per 1000 kcal (Table 2). As a comparison, the vegan diets contained 0.2 μg and ordinary diets 2.7 μg of vitamin B₁₂ per 1000 kcal (Fig 1 and References 6 and 7).

According to FAO/WHO (18), adult men and nonpregnant women are recommended a daily intake of 2 μg of vitamin B₁₂. The minimal requirement of vitamin B₁₂ to prevent clinical signs of vitamin B₁₂ deficiency is 0.1 to $\mu\text{g/day}$ (19, 20).

Intakes of vitamin B₁₂ below requirements might be reflected by subnormal plasma B₁₂

values. As seen in Table 6, all six subjects had values within the normal range. On ordinary diets, subjects have liver stores of vitamin B₁₂ corresponding to about 1000 daily requirements (17). Therefore, intakes below requirements should result in a slow depletion of tissue stores. Vitamin B₁₂ deficiency has occasionally been reported to occur among vegetarians, especially vegans (6). Infants of vegan mothers seem to be a high risk group. Not only are these infants born with small liver stores of vitamin B₁₂, but the milk provided by their mothers is extremely low in B₁₂, containing only one-tenth of the amount usually present in human milk (17).

It has been claimed that vegetarians compensate for their low dietary intake of vitamin B₁₂ by utilizing vitamin B₁₂ produced by the gut flora. Unfortunately, there is no easy way of determining whether endogenous production of the vitamin occurs and, if so, its extent.

The lactovegetarian diet provides considerable amounts of folate. Except from certain viscera (heart, liver, etc), green vegetables are the richest source of dietary folates (17). Although dietary folates are easily destroyed by oxidation and especially during cooking (17), prepared meals of lactovegetarian diet resulted in an average intake of 442 and 359 μg of total folate for men and women, respectively. Thus, the lactovegetarian diet contained twice as much total folate per 1000 kcal as ordinary diets, 182 versus 90 μg (7). The vegan diet, on the other hand, contained 301 μg folate per 1000 kcal, which exceeded the normal intake 3-fold (6) (Fig 1).

The recommended intakes of dietary folates amount to at least 200 μg daily for adult men, as well as nonpregnant women (18). Thus, the lactovegetarians investigated in the present study had daily intakes well exceeding these recommendations. In addition, their whole blood folate concentrations, reflecting their body stores, were within the normal range except for subject DLV-5, who had a subnormal value of 40 nmol/l (Table 6). We have no explanation for this low value for this woman, for her folate intake was as high as the others and her serum B₁₂ was also normal.

Nitrate-nitrite

The lactovegetarian diets contained on an average 6 mg nitrate (as NaNO₃) per 1000 kcal (Table 2). Corresponding figures for the normal and vegan diets were much higher, 18 and 92 mg, respectively. Regarding nitrite, the lactovegetarian diets contained 4.2 mg nitrite (as NaNO₂) per 1000 kcal, whereas corresponding data for the normal and the vegan diets were between 1.5 and 2 mg/1000 kcal (6).

The average nitrate and nitrite intakes expressed per kg body weight and day varied, respectively, between 0.15 and 0.33 mg and 0.08 and 0.25 mg. The acceptable daily intake of nitrate as NaNO₃ and nitrite as NaNO₂ is 5 and 0.2 mg/kg body weight per day, respectively (21). Thus, the lactovegetarian diet contained about twice as much nitrite as normal mixed diets and vegan diets, and the acceptable daily intake of nitrite was slightly exceeded by two subjects DLV-4 (0.22) and DLV-6 (0.25 mg/kg and day).

Clinical and biochemical findings

The subjects were apparently healthy at the time of examination. Their previous diseases and the results from physical examinations are listed in Table 1. The body weights of the lactovegetarians were in the same range as those encountered among vegans (6), but were lower than those among pensioners (7). The same relationship applied to the quotient weight/height³, used as an index of overweight, in the three groups (7). Although the data suggest that vegetarians have a lower body weight, no definite conclusions can be made on the relation between energy intake and body weight owing to the small number of subjects. Dwyer et al (2) found that among 100 vegetarians many were slender and had lost weight when compared with previous maximal weights. Swedish female vegetarians had a somewhat lower body weight than controls and also a smaller amount of body fat (11). The blood pressures of the subjects were normal and even slightly low for their ages. The results from the biochemical analyses of blood and urine are shown in Table 6.

There were no signs of anemia and the

stores of iron, folate, and cobalamin seemed satisfactory, except that subject DLV-5 had a low blood folate. DLV-5 also had a low level of retinol-binding protein. Most subjects instead had supernormal levels of blood folate, which probably was due to the increased consumption of vegetables compared with the reference population. Bergan and Brown (22) found a low transferrin saturation in 14% of vegetarians but no indications of folate or vitamin B₁₂ deficiency.

The concentration of low-density lipoprotein (LDL) among the lactovegetarians was within the normal range, and the LDL/high-density lipoprotein (HDL) ratio was somewhat higher than that observed for vegans (6) and for younger vegetarians (23). Similar data were observed for other vegetarian groups (11, 23, 24). Lower plasma triglycerides than in controls has been found (23), although not in all studies (11). In this study, all subjects had plasma triglycerides within the reference range.

A kinetic study showed that LDL production was significantly lower in vegetarians than in controls (24). Further, the fractional removal of HDL-AI protein was increased, and bile acid excretion was decreased. Nestel et al (24) concluded that the changes in vegetarians probably are due to several dietary factors. In hypercholesterolemic children treated with a vegetarian diet, LDL and HDL decreased, which was attributed mainly to the low cholesterol content of the vegetarian diet (25).

Sacks et al (26) fed 250 g of beef daily for 4 wk to vegetarians, which resulted in increased total plasma cholesterol but not HDL cholesterol. The increase could be ascribed mainly to the lipid components of beef.

The conclusion from the available data is that the low cholesterol content and high P/S ratio of vegetarian diets are important for the decreases in LDL cholesterol observed in several studies.

Among plasma proteins, four subjects had subnormal prealbumin levels; but albumin concentrations were all normal, indicating a normal protein status. Studies in a lacto-ovo-vegetarian community also showed normal plasma concentrations of total protein and albumin (27).

One subject had elevated serum bilirubin, which may be related to a previous episode of jaundice or to carotenoids, which may interfere with the determination. Other tests on liver function were essentially normal. Also concentrations of serum electrolytes were within the reference range. In contrast, Harland and Peterson (27) found several low levels of serum magnesium. Chemical variables on thyroid function were all normal; and were also normal in the subject (DLV-3) with subtotal thyroidectomy. 

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***Public health/Clinical significance
of inorganic chemical elements***

PUBLIC HEALTH/CLINICAL SIGNIFICANCE OF INORGANIC CHEMICAL ELEMENTS

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SUMMARY

Food tables documenting the concentration of various nutrients in individual foods do not provide satisfactory information on the contribution of essential inorganic trace elements by prepared meals. Only direct analysis of the actual food consumed during a 24-hour period can give an accurate estimate of the dietary intake. Employing the duplicate portion technique, we have analysed the concentration of a number of inorganic chemical elements in 882 dietary samples from various population groups in Sweden. The intake of several elements including potassium, magnesium, zinc, copper and selenium, in the normal mixed diets is low when compared with recommended dietary allowances (RDA). Vegetarian diets are richer in these elements than ordinary non-vegetarian diets. Plasma levels of trace elements are often poor indicators of body status. A clinical follow-up of a group of pensioners in Dalby, Sweden, for a period of 10-12 years has not indicated any specific signs or symptoms of trace element deficiencies. Vulnerable groups, namely, children, pregnant women, alcoholics and the elderly, may, however, be more susceptible than the population in general to marginal deficiencies resulting from normal everyday consumption of Western diets.

* * *

Advances in analytical methodology and sophisticated instrumentation have certainly helped to bring about a breakthrough in the field of trace element research during the last two decades. Using powerful analytical techniques, such as atomic absorption spectrophotometry, neutron activation, mass spectroscopy and polarography, researchers have demonstrated the presence in living systems and their food chain of a number of inorganic chemical elements - elements that were considered up till now as contaminants or accumulated material, and as such, of no importance in human health and disease. The latest estimates indicate that most tissues of a healthy adult contain 40-60 of the basic elements represented in the periodic table (Hamilton, 1979; Abdulla et al., 1982a). In studies concerning the health and diseases of man, these are traditionally classified as essential or non-essential according to their role in vital metabolic processes. The bulk of living matter consists of the first eleven elements in the periodic table. Such so-called trace elements as chromium, cobalt, copper, iron, manganese, zinc and selenium constitute somewhat less than 1% of the total human body mass. The number that are known to be essential for life is likely to increase in the future with advances in our knowledge concerning the role of the elements in metabolic reactions.

In spite of the tremendous progress in trace-element nutrition research during the last few decades, the biological roles as well as the minimum requirements of many elements are still hypothetical. Some of these that are present in the human body in very low concentrations (ppb levels) are not thought to have any specific biological function. At the same time, it is generally believed that the minimum requirements of essential chemical elements, including chromium, copper, manganese and selenium, are so low that a pure nutritional deficiency of these rarely occurs in man. On the other hand, the adequacy of a modern all-round Western diet as regards trace elements is presently being debated. Marginal deficiencies of several trace elements have been shown to exist in parts of the populations of industrialized countries (Mertz, 1970).

From a public health point of view, it is important to assure the general population that the intake of all nutrients, including the essential elements, is adequate in the average, normal daily diet. At the same time, the ideal diet should not contain more than the permitted levels of toxic heavy metals. Except for occupational exposure, the major pathway through which the chemical elements enter the human body is via the food chain. Requirements established by controlled balance studies exist only for a few elements. Information concerning the dietary intake of essential inorganic elements from prepared meals is very limited at this time. Further, the data currently available are unsatisfactory, since most of them are based on conventional techniques, such as dietary history and interview studies in combination with food tables. Food tables have limitations when used for the assessment of trace-element intakes. They do not, for example, take into consideration the effect of preparing and cooling, prior to consumption, which often alter the composition of raw food materials. Moreover, food tables have no data for some elements, which occur in very minute amounts in basic foodstuffs.

Another important problem in trace-element nutrition is the diagnosis of deficiency. Severe deficiencies that require immediate medical attention occur only rarely in affluent countries. Such cases are reported occasionally in hospitalized patients receiving total parenteral nutrition (Jeejeebhoy et al., 1977; Mertz, 1981a). On the other hand, there are indications that marginal deficiencies of magnesium, copper, zinc and selenium, are fairly common in parts of the population of industrialized countries (Abdulla et al., 1981c; Mertz, 1981b). A lack of characteristic symptoms and diagnostic techniques is the major reason that marginal deficiencies are not identified at an early stage. Even when the dietary intake is restricted, normal body functions are maintained for a certain period of time by homeostatic mechanisms and by making use of the body reserves. An ideal approach to study the long-term effect of marginal consumption of trace elements is to follow vulnerable groups in the general population over extended periods for possible signs and symptoms of deficiency. Another way of detecting the existence of marginal deficiency rests in therapeutic trials. The response to iron supplementation in iron-deficiency anaemia is a good illustration. Unfortunately, there are no

clinical parameters, such as haemoglobin levels in blood, to correlate the therapeutic response to other trace elements. Changes in the activity of metalloenzymes in blood and other tissues can sometimes be related to the concentration of trace elements. In our own studies, we could show that the activity of delta-aminolevulinic acid dehydratase in red blood cells could be activated by oral supplementation of zinc (Abdulla & Haeger-Aronsen, 1971; Abdulla & Svensson, 1979, Abdulla, 1980; 1981). Similarly, alkaline phosphatase activity in plasma and other biological fluids has been found to be correlated with zinc concentrations (Kirchgessner & Roth, 1980).

At Dalby in Southern Sweden, the Swedish Medical Board supports a unit for primary medical care. Dietary habits and the general health of the population is one of the research projects in this unit, wherein the duplicate portion technique has been standardized for studying the dietary intake of all nutrients in the diet of various population groups in Sweden (Borgström et al., 1975). One group of old age pensioners has been followed for a period of 10-12 years. I shall, in this paper, briefly discuss the technique used to study the dietary intake of essential and toxic inorganic chemical elements (Abdulla et al., 1979a) and the major findings of our follow-up studies.

MATERIALS AND METHODS

The material consisted of 882 dietary, 243 urine and 457 blood samples. The particulars of the participants are shown in Table I.

The duplicate portion technique was employed to collect the daily dietary samples. The duplicate portion was a copy collected visually while eating to represent as exactly as possible the food and fluids consumed during a 24-hour period. The subjects were briefed thoroughly by a trained dietician about the sampling procedure. On the day of sampling, a written protocol was prepared to indicate the rough quantities and types of solid foods and fluids consumed. The material collected during the day was pooled and later homogenized and 100-200 ml of the homogenized material lyophilized. This

Table I : Particulars of the participants (various population groups) included in the Dalby study

Groups investigated	Age range	Sex		No of samples collected		
		M	F	Diets	Urine	Blood
Healthy adults	25 - 60	10	10	140	-	-
Healthy women	30 - 79	-	60	360	180	60
Pensioners	67	17	20	259	-	370
Vegans	49 - 55	3	3	24	24	6
Lactovegetarians	49 - 52	3	3	24	24	6
Children	14 - 15	7	8	75	15	15

material was then extracted using chloroform and methanol to determine the fat-soluble components of the diet. The fat-free powder was then dried, quantitated and used to estimate the concentration of the inorganic elements.

The 60 women, 12 vegetarians and 15 teenagers who participated in our study also collected 24-hour urine samples corresponding to the days of food sampling. From most of the participating subjects, blood samples were drawn at least on one occasion. In the case of the pensioners, blood samples were collected on every occasion of a clinical follow-up. Zinc and magnesium levels were measured in whole blood as well as in plasma, whereas the other elements were determined only in plasma. The concentrations of nickel, chromium, iodine, lead, cadmium and mercury were also measured in the diets of the pensioners.

For analysing the inorganic elements, approximately 0.5 g of the fat-free powder was wet-ashed at below 250°C using concentrated nitric, sulphuric and perchloric acids. The details of the procedure are reported elsewhere (Borgström et al., 1975). Following wet-ashing, the samples were diluted with de-ionized water and kept in the cold room until analysis. For the analysis of calcium and magnesium, the samples were diluted with 2% w/v

lanthanum chloride. Sodium and potassium were analysed by flame photometry; the others were determined by atomic absorption spectrophotometry except iodine which was quantitated by a spectrophotometric method (Abdulla et al., 1979b). A number of 24-hour dietary samples were also subjected to neutron activation for comparison of results with those obtained by other techniques.

The clinical examination in the case of pensioners consisted of a standardized questionnaire and a routine clinical examination, including the analysis of blood and urine. The laboratory data included height, weight, blood pressure, haemoglobin, leucocyte counts, erythrocyte sedimentation rate, blood folate, serum cobalamine, plasma tocopherol, serum vitamin A, serum proteins, serum triglycerides, serum cholesterol, serum T_3 and T_4 , blood glucose and liver function tests. The concentration of zinc, copper, iron, magnesium, calcium and selenium was also measured in the plasma. The extensive clinical and laboratory investigations of the pensioners were undertaken at 2-year intervals for 12 years. These investigations are still being continued in those who are alive.

The material also consisted of 24 hospital diets (breakfast, lunch and dinner). These were included in order to make a comparison of the results by computation employing standard food tables, as well as by chemical analysis. These hospital dietary samples were treated in the same way as the other diets prior to chemical analysis.

Urine samples, whenever collected, were analysed for the estimation of the electrolytes sodium and potassium and nitrogen. We also measured the excretion of calcium, magnesium, zinc and copper in a number of urine samples.

RESULTS

The losses of the inorganic elements during lyophilization and fat extraction were calculated by analysing the fresh homogenates and the fat-free, lyophilized powder. In most cases, the losses were less than 5%. Recovery studies indicated that the wet-ashing procedure resulted in a 5-10%

loss of sodium, potassium and magnesium. For the remaining elements, the losses were less than 5% (Abdulla et al., 1982a). Table II shows the concentration of calcium, magnesium, sodium and potassium in the "common" (the averages of the diets of adults, women and pensioners), vegan and lactovegetarian diets. (The results of the diets from teenagers were not completely ready at the time of preparation of this manuscript). Fig. 1 shows the intake levels of iron, zinc, copper and selenium in the diets as percentages of the recommended dietary allowances levels (RDA) (National Academy of Sciences, Washington, 1980). As can be observed, the concentration of most of the elements in the common diet is low when compared with the RDA levels. This is especially so for potassium, magnesium, zinc, copper and selenium. The vegetarian diets, on the other hand, have much higher concentrations of these elements when compared with those of the common diet. The selenium content in the vegan diet is extremely low, whereas the lactovegetarian diet has the highest concentration of all of the element. The lactovegetarian diet, on the whole, is richer in most trace elements than the so-called common diet, but slightly poorer than the vegan diet except for selenium. Mercury, cadmium and lead in the pensioners' diets is below the permitted levels proposed by the FAO/WHO (Expert Committee on Food Additives, 1972); chromium, nickel and iodine intakes, on the other hand, are far more than adequate when compared with the present recommended levels (RDA, 1980).

The results obtained using food tables and by chemical analysis of the hospital diets showed that the computed data, in general, gave higher values than those obtained by chemical analysis. The food tables normally do not have data for the concentration of several trace elements such as copper, chromium and selenium, and as such are not suitable for comparative studies. In the present investigation, we only compared the concentration of iron, calcium, sodium and potassium. Fig. 2 shows the data for the concentration of iron obtained using food tables and by chemical analysis.

Table III shows the results of the analysis of plasma and urine samples for the concentration of calcium, magnesium, zinc and copper. Those for sodium, potassium, and in some cases, selenium and chromium, are not tabulated here. In general, most of the values obtained in the present investigation lie within the normal range.

Table II : Concentration (mean and range, mmol/l) of sodium, potassium, calcium and magnesium in the common and vegetarian diets

Elements	Types of diets		
	Common	Vegan	Lactovegetarian
Sodium	102 (16-294)	96 (23-168)	104 (25-188)
Potassium	52 (15-93)	103 (51-163)	125 (53-160)
Calcium	17 (5-67)	16 (3-33)	23 (12-41)
Magnesium	9 (4-20)	22 (10-35)	18 (12-32)

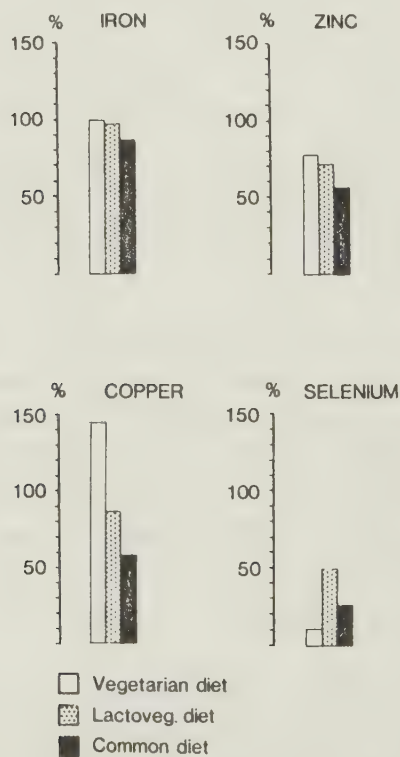


Fig. 1 : Concentrations of iron, zinc, copper and selenium in the diets as percentages of the RDA levels.

Table III : Concentration of calcium, magnesium, copper and zinc in plasma and urine (mean and SD, mg/l)

Groups		Calcium	Magnesium	Copper	Zinc
Lactoveg.	plasma	92.2 ± 6.7	17.2 ± 1.9	0.950 ± 0.170	0.91 ± 0.16
	urine	99.5 ± 60.9	67.1 ± 20.1	0.038 ± 0.015	0.37 ± 0.24
Vegans	plasma	---	23.1 ± 1.2	1.090 ± 0.240	1.14 ± 0.31
	urine	56.9 ± 18.1	80.5 ± 24.8	0.040 ± 0.021	0.32 ± 0.11
Pensioners	plasma	94.5 ± 9.7	18.4 ± 1.7	1.280 ± 0.370	0.80 ± 0.12
	urine	---	---	---	---
Children 15 ys-old	plasma	133.0 ± 5.7	19.1 ± 1.3	1.000 ± 0.130	1.15 ± 0.11
	urine	---	---	---	---

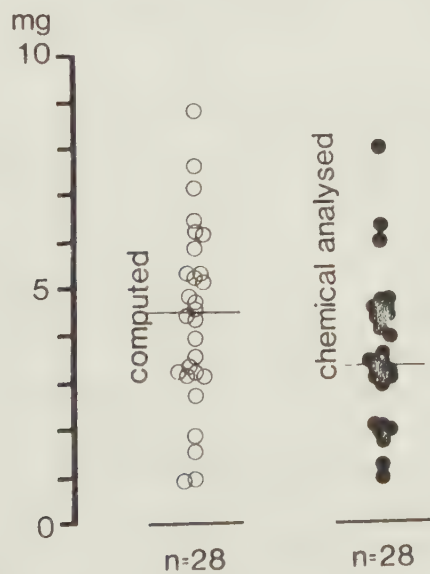


Fig. 2 : Comparison of the concentration of iron computed using food tables and that obtained by chemical analysis (hospital diets).

DISCUSSION

In the past, food tables documenting the composition of individual food items have been extensively used for mass surveillance of food quality, especially with regards to the macronutrients proteins, fat and carbohydrate. The tables are generally based on the analysis of raw foodstuffs and, as such, may not be accurate for estimating the intake of micronutrients, especially the trace elements, from prepared meals ready for consumption. Besides, they do not provide values for chromium, selenium, manganese and arsenic. Development of the duplicate portion technique has eliminated many such problems. One major advantage of the technique is the possibility to store dietary samples for an unlimited period of time thus permitting later analysis for any particular element of interest that has been omitted at the time of the first investigation. This is pertinent because more and more elements in the periodic table are gaining importance in the field of human nutrition.

Because the results of the chemical analysis generally show lower values than those computed using food tables, the duplicate portion technique has been criticized in that it may not represent what people really consume on a day-to-day basis. This aspect has been investigated in detail in the study of the 60 women (Thulin et al., 1980; Abdulla et al., 1981b) in whom the urinary excretion of electrolytes and nitrogen was measured on two different occasions without the simultaneous collection of dietary samples. On a third occasion after an interval of two years from the first, these women collected six daily portions of food and three 24-hour urine samples corresponding to the days of food collection. Once again, we analysed the intake levels and excretion of sodium, potassium and nitrogen. There was no significant difference between the three investigations. Thus, it appears that the food collection technique did not influence the pattern of normal eating habits during the period of our investigations. Analysis of hospital diets by computation using food tables and by chemical analysis also show that the latter approach gives lower values than the computed data. A close look at the available literature indicates that the previous estimate of trace-element intakes was based on rough estimates from the levels found in the raw food materials. Our results from different series of dietary samples

are reproducible and in agreement with the results of others who employed the same technique (Smith et al., 1982).

Our most important finding is the low content of several essential elements in the so-called common diet as compared with the recommended dietary allowance (RDA) levels. Similar results have been found in the diets of the general population in other affluent countries (Klevay et al., 1979; Smith et al., 1982). Overconsumption of highly refined food items may partly contribute to the low intake level of some of the elements, such as zinc, copper and selenium (Underwood, 1977). The vegetarian diets consisting mainly of unrefined foodstuffs, indeed, have a higher content of most essential trace elements; however, they may not be biologically fully available for absorption. High-fibre content in the diet can reduce the absorption of several trace elements (Sandstead et al., 1976) and vegetarian diets are fairly rich in fibre (Abdulla et al., 1981a). More detailed and long-term studies are necessary to evaluate whether vegetarian diets are a better or an inferior source of bioavailable trace elements than the normal mixed diets. Such studies may be conducted in populations such as the Hindu Brahmins in India who, because of religious beliefs, have been vegetarians for generations.

Although the plasma levels of the elements investigated are found to be in the normal range, it is difficult to interpret the results with regard to the body status of these elements. At present, no single laboratory parameter indicates the body status of most of the inorganic elements. Traditionally, the concentrations are measured in plasma or serum, for it is a simple, fast and inexpensive method. Plasma or serum levels, however, may not be good indicators for many, including zinc, copper, magnesium and iron, as most of these are intracellular (Abdulla, 1982). Moreover, a low or high level of an element in the plasma or serum may not always indicate a dietary deficiency or excess of the element in question. Very often, changes in plasma levels are due to a shift of the element from the transport media to other body compartments (Abdulla, 1982). A wide range of conditions, such as acute and chronic infections, inflammatory diseases, burns and myocardial infarctions, are known to influence the plasma levels of trace elements (Beisel et al., 1976; Powanda, 1982). A number of pharmaceutical drugs that

are in common use are also known to influence the plasma levels of several trace elements (Weismann, 1980).

For reliable information on the body status of trace elements, direct measurements in vivo - whole-body counting using radioisotopes and analysis of vital biopsy materials : viz. liver, kidney, muscle and bone sample - are indicated. For routine purposes, these methods are, however, not practicable. So much so, that until simple or sensitive methods for the assessment of trace-element status are developed, dependence will be on indirect measurements of plasma, urine, hair and nail samples. To assess the body status of trace elements, it is advantageous sometimes to combine the analysis of urine and plasma samples. Analysis of nucleated tissue such as leucocytes has recently been found to be a reliable indicator of body status of trace elements including zinc when compared with that of plasma levels alone (Whitehouse et al., 1982). Because the turn-over rate of trace elements in leucocytes is fast, deficiency states may be reflected at an early stage, if the cellular concentration of these elements is accurately measured. Specific enzyme studies may be another possibility for assessment of trace elements status in the future.

As pointed out earlier in this paper, the right approach to detect a deficiency of trace elements is to follow clinically the vulnerable groups in the general population, namely, children, pregnant women, alcoholics and the elderly, for prolonged periods of time. This may not be possible in many countries due to economic and social problems. In the present study, we have followed the health status of the pensioners in Dalby, Sweden, for 10-12 years. Although the elderly people of industrialized countries constitute almost 25% of the total population, this group may not be the ideal one to follow with regards to trace element deficiencies. With advancing age, a gradual deterioration of many physiological functions are to be expected. Energy requirements decline gradually during the last decades of life. Deficiencies in the dental status and digestive problems certainly influence the dietary habits of the elderly. Moreover, elderly people are more often subjected to various drug therapies and this may influence trace-element metabolism (Abdulla et al., 1977).

Of the 37 Dalby pensioners clinically followed during the last 10-12 years, 27 are still alive today. From the clinical evaluations, which always included an elaborate laboratory data, we are unable to single out any signs or symptoms that can be attributed to the low intake of the elements we investigated.

This brings up the very important question concerning the adequacy of the present RDA values. The RDA values are subject to revision from time to time and it is often difficult to draw conclusions regarding the consequences of low intake levels of many essential elements. Moreover, there are no internationally acceptable RDA levels for many trace elements. Growing children and/or pregnant women are a more ideal group to study the effect of marginal intakes of trace elements. These groups are more susceptible to marginal deficiencies of trace elements, such as iron, zinc and copper, than the rest of the population (Mertz, 1970; Hambidge et al., 1972; Jameson, 1976; Abdulla et al., 1982b).

Another approach in the diagnosis of marginal deficiencies of trace elements rests in therapeutic trials. Many of the essential elements, for example, zinc, copper, iron and selenium, are non-toxic in therapeutic doses. Studies in Egypt and Iran on zinc deficiency and in China on selenium deficiency are good examples illustrating the positive effect of therapeutic trials (Prasad, 1976; Chen et al., 1980). In pregnant women with very low plasma zinc levels, oral zinc supplement had beneficial effects when compared with a control population (Jameson, 1976). The effect of zinc during pregnancy in humans is very similar to that which has been observed in experimental animals (Hurley & Swenerton, 1966; Mathur, 1978). Safe enrichment of table salt with iodine and the dramatic response of nutritional goitre is a classic example of therapeutic nutrition. Similarly, the response of iron-deficiency anaemia to iron supplementation has been recognized for centuries. It might be worthwhile promoting similar strategies of fortification and supplementation programmes to deal with deficiencies of other trace elements such as zinc, copper, manganese and selenium. During the last few decades, more attention has been directed to studies in animals than in man to elucidate the health effects and interactions of trace elements. It is perhaps more auspicious now to concern ourselves with human health problems in this context.

CONCLUSIONS

The duplicate-portion food sampling technique is a simple and reliable procedure to study the dietary intake of essential and toxic trace elements. Collection of 24-hour urine samples may provide additional information concerning the adequacy of the sampling. Chemical analysis of the dietary intake of the population groups investigated gave lower values than are obtained by computations using food composition tables; hence, their use may not be ideal for estimating the dietary intake of trace elements from prepared meals.

Chemical analysis indicated lower concentrations of the elements potassium, magnesium, zinc, copper and selenium in the ingested diets when compared with the RDA values. Vegetarian diets, however, had higher concentrations of these elements than non-vegetarian diets; the relatively high content of raw food materials in the vegetarian diets may be one reason. Nevertheless, it is not clear whether these elements are biologically available.

At present, there are no suitable techniques for the early diagnosis of marginal deficiencies of trace elements. Plasma levels are poor indicators of body status. A clinical follow-up study of elderly pensioners at the Dalby Health Center, Sweden, for a period of 10-12 years did not reveal any specific signs or symptoms of a deficiency of any trace element. It is imperative to direct research to developing simple and sensitive techniques for the assessment of trace-element status in vulnerable groups, such as children, pregnant women, alcoholics and the elderly.

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	I	II							
1									
2	Li	Be							
3	Na	Mg							
4	K	Ca	Sc	Ti	V	Cr	Mn	Fe	
5	Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	
6	Cs	Ba	La*	Hf	Ta	W	Re	Os	
7	Fr	Ra	Ac**						

H

*Lanthanides

**Actinides